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RESPIRATION AND OXIDASE AND CATALASE ACTIVITY OF APPLES IN RELATION TO MATURITY AND STORAGE [1]

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INTRODUCTION

Certain physiological changes that are taking place in fruit at the time of harvest continue after it is placed in cold storage. The immediate effect of low temperature is to retard these processes. If all were retarded equally, it might be supposed that the fruit would continue to ripen normally, but at a much slower rate. If, however, the different processes should be unequally retarded, then various disorders might result. That many of the physiological diseases of fruits in storage may be due to an unbalanced condition brought about by an unequal retardation or stoppage of the various life processes by low temperature appears probable. The failure of Bosc or Comice pears to ripen normally after prolonged storage seems almost certainly to be caused in this manner. The enzymes in the pears that change protopectin to soluble pectin no longer function adequately and the fruit remains hard and unpalatable even when external conditions are favorable for ripening. That all the life functions have not ceased is readily shown by the continued production of carbon dioxide. Soft scald, scald, and break-down of apples, and core break-down of pears are other cold-storage disorders that may have their origin in an unequal slowing down of the various life processes.

If all these processes are not retarded equally, one might suppose that the more rapidly they are taking place at the time of storage the greater would be their divergence from normal and the more serious the results. There is some evidence to support this supposition. If Jonathan apples are delayed at high temperature between harvest and cold storage, the life processes, as measured by carbon dioxide production, are speeded up, and the fruit is also more subject to soft scald when stored at 32° F. A more striking example, probably caused by unequal retardation of different processes may be observed with Bartlett pears. If pears of this variety are delayed at warm temperatures for a few days until the ripening processes are well under way and are then stored at 40°, large amounts of acetaldehyde are produced in the tissues, accompanied by a disagreeable odor, excessive scald, and core break-down.

In previous work (*4*, *5*) [2] in which various physiological factors connected with the development and maturity of Bartlett pears were studied, it was shown that the oxidase activity of the fruit decreased throughout the growing season. The curves for catalase and respiratory activity were U-shaped. The low point on these curves occurred at or near the time the fruit should be harvested for best storage and

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[2] Italic numbers in parentheses refer to Literature Cited, p. 470.

dessert quality. These results might indicate that the rate of metabolic activity at the time of harvest and storage influenced the subsequent behavior in storage.

The present work was undertaken to determine whether there is a period at which the various life processes in apples are at a minimum and during which the fruit could be harvested and stored with less danger from the disorders that often develop in storage. The indexes of metabolic activity used were respiration and oxidase and catalase activity. These studies, extending over two seasons, were made at Wenatchee, Wash. For a review of the literature on respiration and enzyme activity, the reader is referred to the publications previously cited (*4*, *5*).

MATERIALS AND METHODS

In order that the fruit throughout the season might be uniform and representative of the changes normally occurring as the fruit matures, representative trees in good vigor were selected, and the fruit was taken from these trees throughout the season. The maturity range included fruit sampled from early July to November. Because of factors over which the writers had no control, it became necessary during the season of 1938 to change the orchard from which the Delicious and Golden Delicious varieties were taken. Fruit of these two varieties harvested on September 6 and thereafter was taken from a different orchard, located in a slightly later maturing district, from those harvested previous to that time. The other varieties were taken from the same trees throughout the season, and the Jonathans from the same trees throughout both seasons.

The methods used in determining the rate of respiration and the catalase activity were the same as those used previously (*5*). In 1937 the oxidase activity was determined iodometrically by the method described by Guthrie (*9*) and in 1938 by that described later by Ezell and Gerhardt (*6*). The rate of growth or weight increase was determined by the average weight of representative fruits taken for the carbon dioxide determinations at each period of sampling. The rate of respiration is reported as milligrams of carbon dioxide per kilogram per hour, catalase as cubic centimeters of oxygen liberated in 5 minutes by 5 cc. of juice, and oxidase as cubic centimeters of N/100 sodium thiosulfate per 2 cc. of juice.

EXPERIMENTAL DATA

RATE OF RESPIRATION AFTER HARVEST

Early in the work it became evident that the rate of respiration of the fruit changed rather rapidly after harvest. The rate and direction of this change were not uniform throughout the season. Some delay after harvest is inevitable. A representative sample must be selected from the trees, the fruit must be brought to the laboratory and placed in the respiration chamber, and the chamber must be sealed and cleared of the carbon dioxide normally present in the air. Moreover the temperature of the fruit at harvest will vary with the air temperature, and it should be brought to room temperature before carbon dioxide determinations begin. Throughout both seasons the fruit was harvested between 8:30 and 9:00 a. m. and all respiration determinations were made at 67° F. unless otherwise specified. Where

cold storage is specified, a temperature of 32° was used. In table 1 the rate of respiration of Jonathan apples at 67°, shown for various periods after harvest, changes rather rapidly. The amount of change during the first 5 hours is unknown but doubtless is appreciable. No determinations were attempted during this period, since 5 hours after harvest was believed to be the minimum time necessary under the conditions of these experiments for setting up the respiration chambers, clearing the container of the carbon dioxide normally present in the air, and bringing the fruit to the temperature of the storage room. When the differences between the temperatures of the fruit and the storage room were large, as on October 18 and 20 and November 7, even more time might have been desirable.

TABLE 1.—*Rate of respiration of Jonathan apples at 67° F. during various periods after harvest*

Date	Period after harvest	Respiration as CO_2 per kilogram per hour	Date	Period after harvest	Respiration as CO_2 per kilogram per hour
	Hours	*Milligrams*		*Hours*	*Milligrams*
Sept. 8..	6 and 7	19.12	Sept. 9..	32 to 34, inclusive	15.14
8..	12 to 14, inclusive	17.20	10..	49 to 51, inclusive	15.47
9..	25 to 27, inclusive	15.55	11..	74 to 76, inclusive	16.87

Figure 1 shows graphically the rate of respiration after harvest throughout the growing season of the four varieties of apples studied during 1938. In arriving at the values plotted, the carbon dioxide determinations began at the end of 5 and 24 hours and usually ran for 3 hours. The values plotted are the average for the 3 hours. These values show that the direction and the amount of changes were not uniform throughout the season and that both may vary with the variety. However, in general it may be said that during the early part of the growing season the rates of respiration of the varieties studied decreased in the period between the 5-hour and 24-hour determinations. During the middle part of the season an increase took place during this time in all the varieties except Jonathan, and in the latter part of the season an increase occurred in all varieties during this period.

These results are of interest because they show that the time elapsing between harvest and the first determination of respiration may alter considerably the shape of the early part of the respiratory curve, and unless the elapsed time is known and kept constant, erroneous conclusions may be drawn when comparing different maturities or varieties. Also, if the complete picture of the respiratory curve of fruit in storage is to be obtained, it is necessary that determinations be made at shorter periods after harvest than has sometimes been done in the past.

The air temperature before harvest might be expected to influence the rate of respiration to some extent. If so, the effect seems to have been largely lost, or relegated to a position of minor importance, within 5 hours. The minimum temperature the night before harvest was approximately 45° F. on August 8, September 8, and October 6; yet on August 8 and September 8 the respiration of Jonathan apples decreased between the 5-hour and the 24-hour determination and

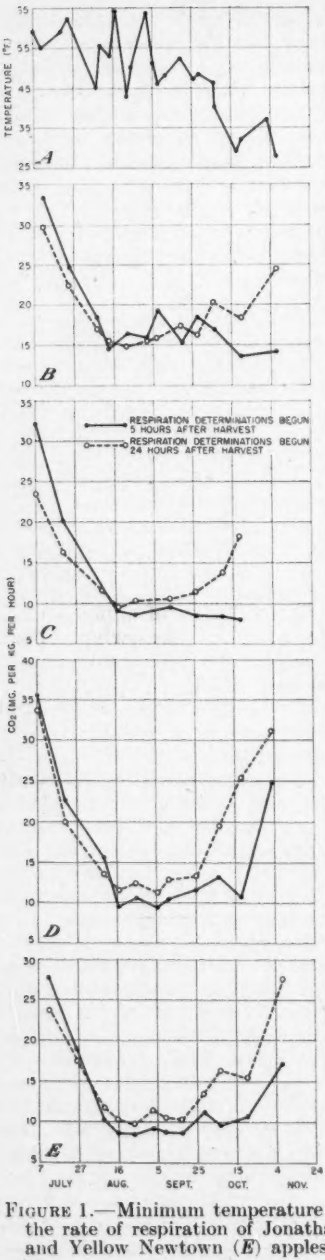

FIGURE 1.—Minimum temperature on night before harvest (*A*) and changes in the rate of respiration of Jonathan (*B*), Delicious (*C*), Golden Delicious (*D*), and Yellow Newtown (*E*) apples during the 1938 growing season.

increased on October 6. Respiration of Yellow Newtown increased during this time on all dates, but the amount of increase was much greater on the latter date. Tests showed that the internal temperature of the fruit had reached the temperature of the storage within 5 hours under the conditions of these experiments and thus precludes the possibility that the difference in respiration was due to a difference in the temperature of the fruit.

In order to determine the amount of variation to be expected in sampling and in the measurement of the respired carbon dioxide, triplicate lots of Yellow Newtown apples were harvested on August 8 and the rate of respiration was determined. These results (table 2) may be taken as an indication of how closely the composite samples used in the respiration experiments reflect the rate of respiration of all the fruit on the tree. Their uniformity precludes the possibility of the wide differences in respiration during the season being due to errors in sampling and in the measurement of the respired carbon dioxide.

RATE OF RESPIRATION AND ENZYME ACTIVITY DURING THE GROWING SEASON

During 1937 Jonathan and Winesap apples grown at elevations of 850 and 2,400 feet were used. Samples were taken at intervals from July 14 to November. On November 2 approximately 90 percent of the Winesaps had dropped, and sampling of this variety for respiratory activity was discontinued. The last sampling of Jonathans for respiratory activity was taken November 15, approximately 8 weeks after commercial harvest. One additional sampling for enzyme activity was made where fruit was available. Figures 2 and 3 show the rate of respiration and oxidase and catalase activity of the two varieties.

TABLE 2.—*Rate of respiration in triplicate 36-fruit lots of Yellow Newtown apples*

Date	Period after harvest	Respiration as CO_2 per kilogram per hour		
		Lot A (2,792 gm.)	Lot B (2,568 gm.)	Lot C (2,758 gm.)
	Hours	*Milligrams*	*Milligrams*	*Milligrams*
Aug. 8	7 to 9, inclusive	10.08	10.11	10.61
9	24 to 26, inclusive	11.57	11.91	11.65
9	27 to 29, inclusive	11.65	12.00	11.48

With Jonathans the rate of respiration decreased in the early part of the season and reached a minimum about the middle of September at approximately the time the fruit would be harvested commercially. The rate of respiration then increased as maturity advanced and finally decreased late in the season. The final decrease started when vascular water core became evident in appreciable amounts; slight water core was noticeable 10 days earlier at the higher elevation. Oxidase activity decreased until past the commercial harvest season and then increased slowly. This increase coincided with the development of water core in the tissue and may have been caused by the accumulation of anaerobic respiratory products in the waterlogged tissues; alcohol has been shown to exert a stimulatory action on oxidase activity (*5*). Catalase activity increased throughout the season except at the last sampling at the low elevation, at which time the fruit was showing medium to severe tissue and vascular water core.

The Winesap variety behaved similarly to Jonathan. The respiration decreased at first and later increased; oxidase activity decreased and then increased as maturity advanced. The Winesap watercored severely and dropped from the tree, so that it was impossible to continue the test much past the commercial harvest season.

Since fruit grown at 850 feet and 2,400 feet showed the same general trend in both varieties, only fruit grown at 850 feet was used in 1938, and four varieties (Jonathan, Delicious, Golden Delicious, and Yellow Newtown) were studied. Jonathan and Golden Delicious are susceptible to soft scald; Delicious and Yellow Newtown are not. However, Delicious is susceptible to storage scald, especially if harvested before it is fully mature. Beginning early in July, respiration and oxidase and catalase activity of the four varieties were followed during the remainder of the season. The results are plotted in figures 4 to 7.

It will be seen from figures 4 to 7 that at the beginning of the test the rate of respiration was comparatively high in all varieties, but that it decreased rapidly, approaching a minimum around the middle or latter part of August, several weeks before the fruit was ready to be harvested commercially. Respiration remained near the minimum for several weeks, and in the case of Jonathan and Delicious for the rest of the season, if the rate beginning 5 hours after harvest is taken as the accepted one. In Yellow Newtown and Golden Delicious, however, respiration increased in the latter part of the season. The respiration as determined 24 hours after harvest increased in all varieties late in the season, and the curves based on the data are U-shaped.

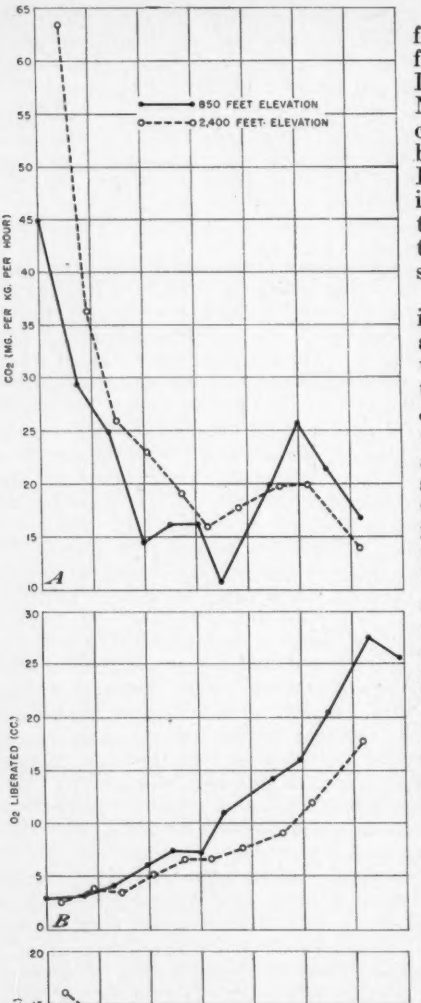

FIGURE 2.—Rate of respiration beginning 5 hours after harvest (*A*) and catalase activity (*B*) and oxidase activity (*C*) during the growing season of Jonathan apples grown at two elevations, 1937.

Oxidase activity decreased from early July until the fruit was ready for harvest. In Jonathan and Yellow Newtown oxidase activity continued at this low rate, but in Delicious and Golden Delicious oxidase activity increased near the end of the sampling period, after the commercial harvest season was over.

Contrary to its behavior in Bartlett pears (*4*), catalase activity started low in all varieties and increased as the season progressed. In Jonathan and Yellow Newtown the increase was slow and uniform throughout the season, but in Delicious and Golden Delicious the rate of increase was more rapid in the latter part of the season. This period of rapid increase started before the fruit was commercially mature and continued as long as the fruit remained on the tree. The magnitude of catalase activity late in the season was also much greater in Delicious and Golden Delicious than in Jonathan and Yellow Newtown.

SEASONAL DIFFERENCES IN RESPIRATION AND ENZYME ACTIVITY

Jonathan apples from the same trees were used during two seasons. The rates of respiration and the oxidase and catalase activity are shown in figure 8. It will be seen that although there are variations, the general trends are similar for the 2 years. Catalase started low and increased throughout the season. At the time of

the first sampling the activity of this enzyme was about the same in the 2 years, but in 1937 the increase was more rapid and reached a greater height. The oxidase activity decreased during both seasons, and, although the curves indicate a close correlation between the two seasons, in reality the activity in 1938 was relatively less, as the ascorbic acid substrate used that year is more readily oxidized than the glucose-derivative substrate used the previous year (*6*). The increase that occurred late in the 1937 season was not so evident in 1938. Respiration started at a high rate and decreased rapidly until mid-August, and although there was some variation between sampling dates, in general the respiratory activity remained near this level in 1938. In 1937 respiratory activity dropped in late September and then increased more than doubling the minimum rate within 30 days. After this rise it decreased about as fast as it had increased. This increase coincided with, or followed closely, the appearance of water core, which became evident in early October and was medium to severe by October 22. The following year the water-cored condition was not evident quite so early, but it had become severe by October 20, without resulting in an

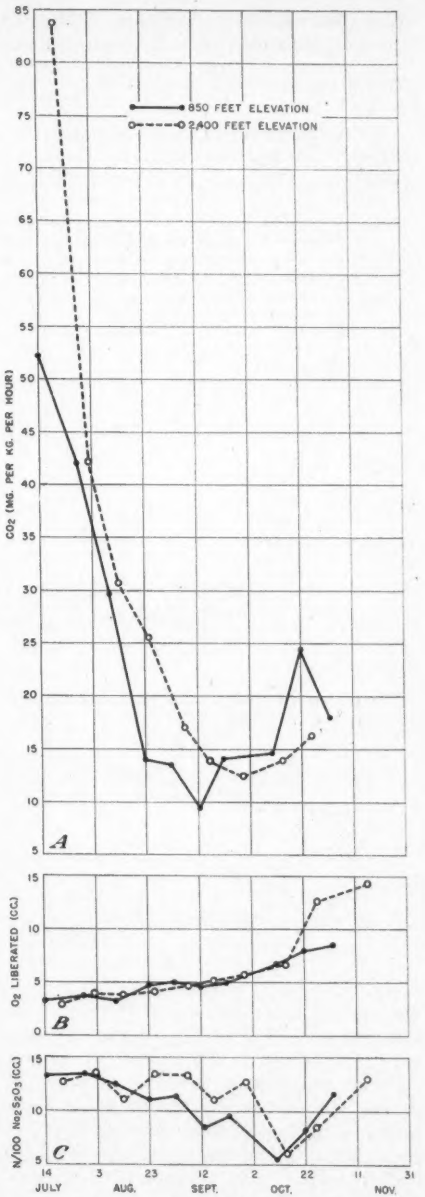

FIGURE 3.—Rate of respiration beginning 5 hours after harvest (*A*) and catalase activity (*B*) and oxidase activity (*C*) during the growing season of Winesap apples grown at two elevations, 1937.

increased rate of respiration. Thus the effect, if any, of the water core on respiration is not clear.

EFFECT OF MATURITY AT HARVEST ON RESPIRATORY AND ENZYME ACTIVITY IN STORAGE

To determine the effect of maturity on the respiratory and enzyme activity at harvest and on their subsequent behavior in storage, Jonathan, Delicious, Golden Delicious, and Yellow Newtown apples

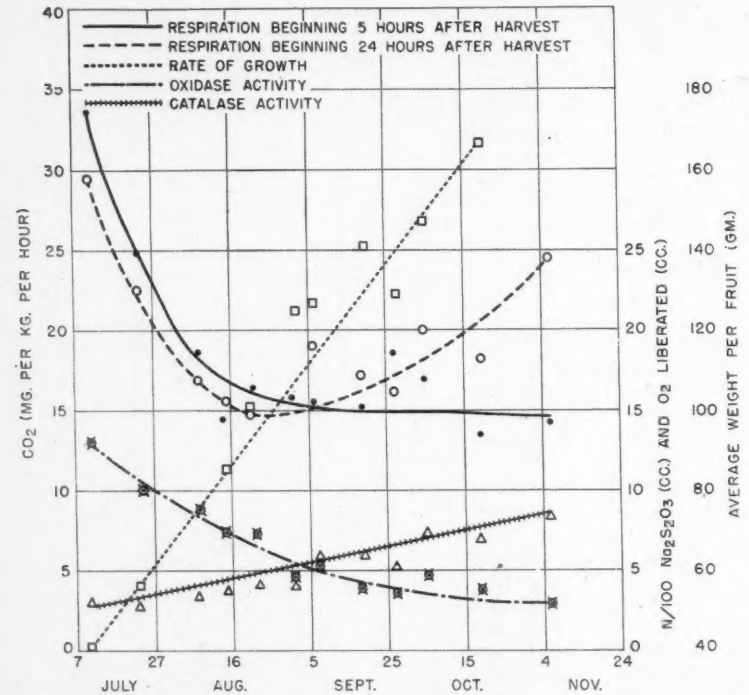

FIGURE 4.—Respiration rate, oxidase and catalase activity, and rate of growth of Jonathan apples during the growing season, 1938.

were picked at the beginning of, toward the middle of, and at or near the end of the commercial harvest season and placed immediately in storage at 32° F. The dates of harvesting and pressure tests are given in table 3. Figures 9 to 12 show the rate of respiration and enzyme activity in storage of fruit of the three maturities. Fruit picked for storage at the beginning and near the middle of the commercial harvest season showed little difference in rate of respiration in storage, although that of the second picking showed a slightly higher initial rate (determined 1 day after storage at 32°) and in general was slightly higher in storage. The third picking, made after most of the fruit had been picked commercially, showed definitely

greater initial activity and also a greater activity in storage than the two earlier pickings. Fruits of this maturity also showed the greatest range in respiration in storage. The greatest catalase activity and the lowest oxidase activity in storage were usually found in the fully matured fruit.

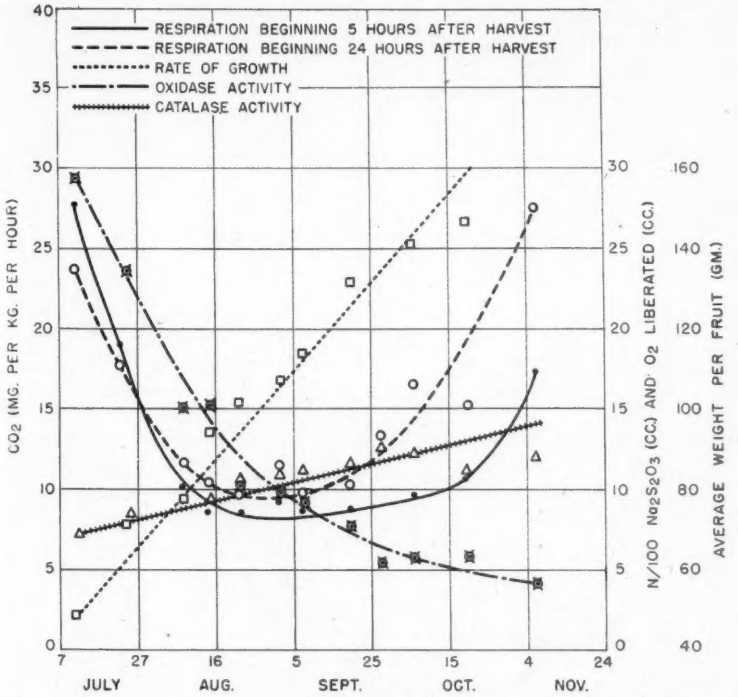

FIGURE 5.—Respiration rate, oxidase and catalase activity, and rate of growth of Yellow Newtown apples during the growing season, 1938.

TABLE 3.—*Date of picking and pressure test of fruit stored at 32° F. immediately after picking*

Variety	Picking		Pressure test	Variety	Picking		Pressure test
	Order	Date			Order	Date	
			Pounds				*Pounds*
Delicious	First	Sept. 12	17.1	Jonathan	First	Sept. 8	17.6
	Second	Sept. 26	16.1		Second	Sept. 20	16.3
	Third	Oct. 18	14.8		Third	Oct. 20	15.7
Golden Delicious	First	Sept. 12	17.4	Yellow Newtown	First	Sept. 16	20.5
	Second	Sept. 26	16.0		Second	Oct. 6	18.0
	Third	Oct. 18	14.0		Third	Oct. 20	17.8

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DISCUSSION

Results from this study indicate that there is no sharply defined period at which the metabolic activity of apples, as indicated by respiration and oxidase and catalase activity, is at a minimum and at

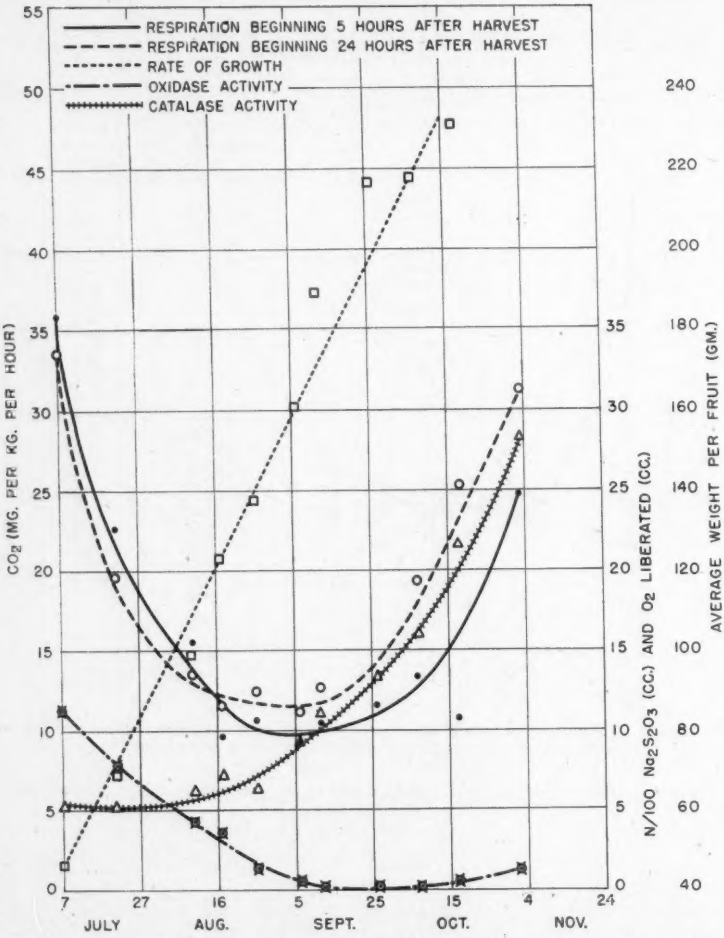

FIGURE 6.—Respiration rate, oxidase and catalase activity, and rate of growth of Golden Delicious apples during the growing season, 1938.

which the fruit may be harvested and stored with least danger from physiological disorders. As the fruit matures the values for the rate of respiration, as determined 24 hours after harvest, form a U-shaped curve, but the base of the curve is rather broad and is reached before the fruit is ready for harvest.

In the present study it was shown that the time elapsing between harvest and the measurement of the respired carbon dioxide affects the rate of respiration and may change the shape of the respiration

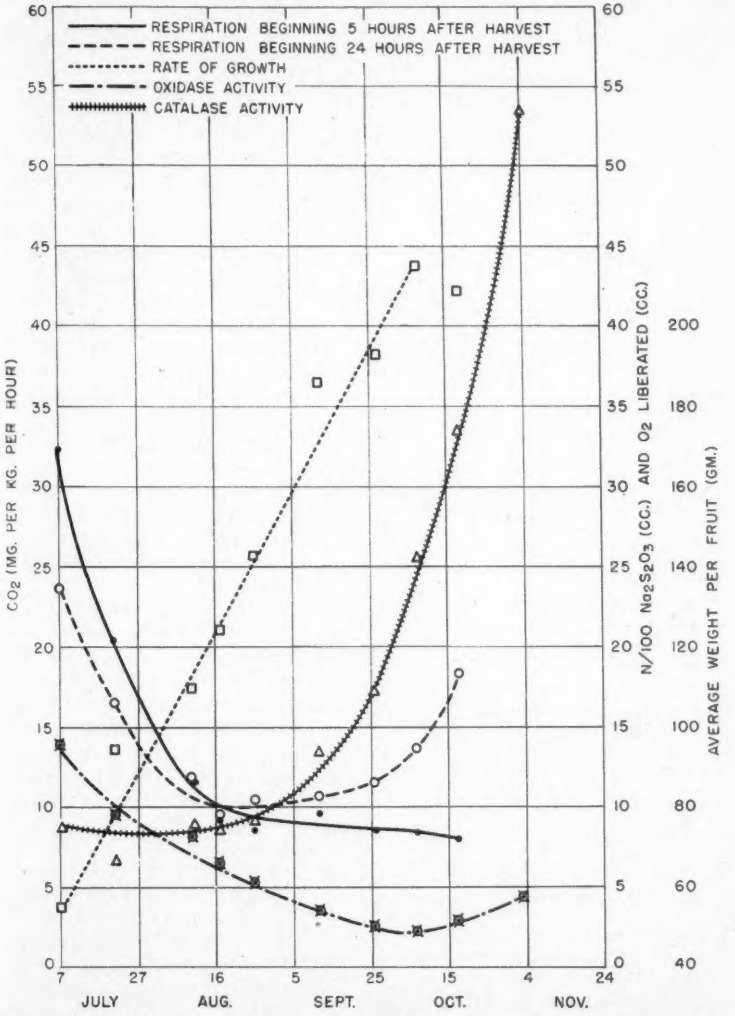

FIGURE 7.—Respiration rate, oxidase and catalase activity, and rate of growth of Delicious apples during the growing season, 1938.

curve. If the measurement is begun at the end of 5 hours after harvest, Delicious does not show the increase in the latter part of the growing season, and consequently the curve for the values is not U-shaped (fig. 7). In Golden Delicious (fig. 6), in Yellow Newtown

(fig. 5), and in Winesap (fig. 3) a definite increase is noted even after 5 hours. With Jonathan an increase is noted in one season (fig. 2) and none in the other (fig. 4). But if the period elapsing is extended through 24 hours, definite U-shaped curves are observed in all cases. Even where an increase is observed after 5 hours, the increase is much greater after 24 hours. Gore (*7*) reported that there was no stimulus in rate of respiration of peaches due to picking from the tree. If this is also true for apples, then it seems logical to assume that the sooner after harvest the measurement is made the more closely it should represent the rate at harvest, and it is possible that if the determination were made immediately after harvest no increase during the season would be noted in any variety. However, a U-shaped curve, determined 5 hours after harvest, has previously been reported for Bartlett pears (*5*).

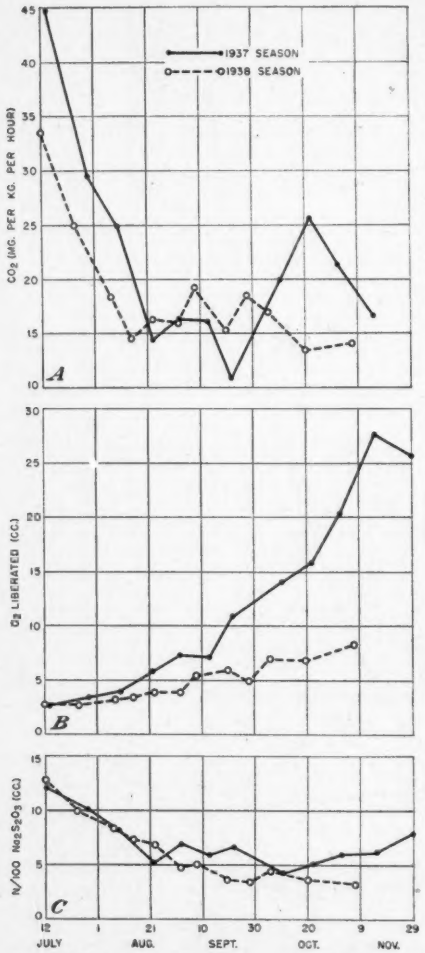

FIGURE 8.—Seasonal differences in rate of respiration (*A*), in catalase activity (*B*), and in oxidase activity (*C*) of Jonathan apples.

Other workers also have reported a decrease in respiration during the growth of the fruit with a later increase as the fruit approached maturity. Kidd (*1*) stated that respiratory activity of apples is greatest at the beginning of fruit formation, falls to about one-fifth at the end of cell division (fruit about the size of a walnut), and continues to decrease slowly during cell enlargement. During maturation a critical change in respiratory activity occurs, and there is a sharp rise followed by a slow decline during senescence. Gustafson (*8*) found that with tomatoes the carbon dioxide production reached a minimum about the time of cessation of growth and then increased to a maximum when the fruits were orange to red in color. Finally there was a subsequent decrease "as the tomato approaches a condition of a mixture of carbohydrate and

water." In his work approximately one-half hour intervened between harvest and the beginning of the respiration determinations. Matsumoto (*15*) reported that with peaches the minimum rate of carbon dioxide production occurred at the period of maximum growth

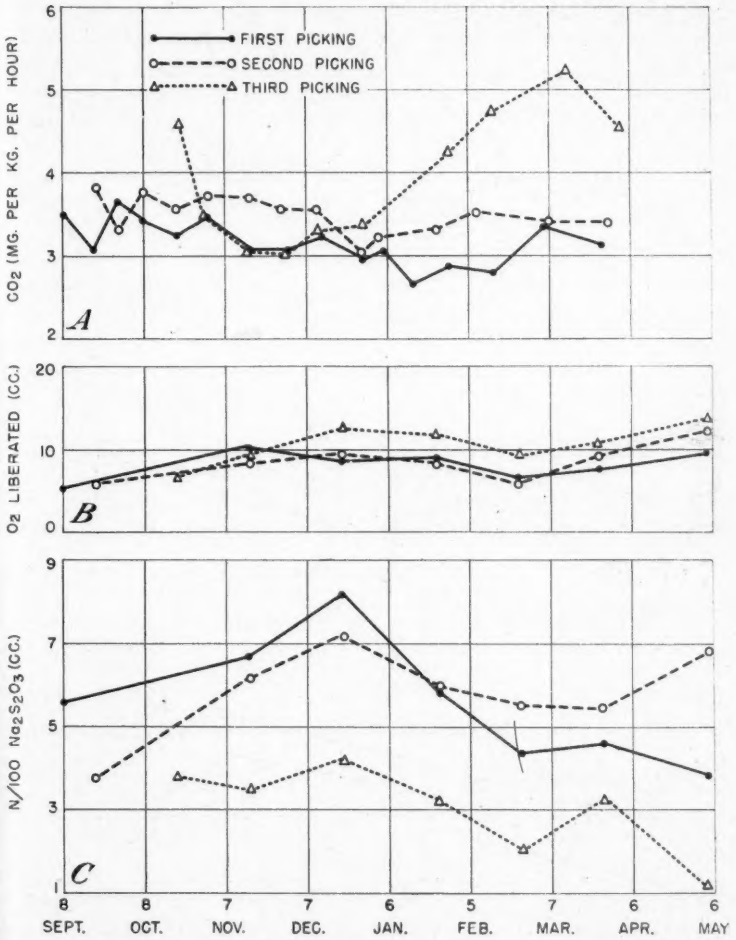

FIGURE 9.—Effect of maturity at harvest on rate of respiration (*A*), on catalase activity (*B*), and on oxidase activity (*C*) of Jonathan apples during cold storage.

and then increased until the fruit was overripe, 15 or more days later. Unfortunately, he did not state how soon the respiration studies were started after the fruit was picked. He also reported that pears and apples showed a similar trend in respiration, but that with Satsuma oranges the rate of respiration was lowest in the least mature fruit and

gradually increased as the fruit approached maturity. However, since his data for Satsumas covered only the last 3½ months of the growing season of the fruit, it appears quite probable that had he extended his observations over a greater part of the growing period

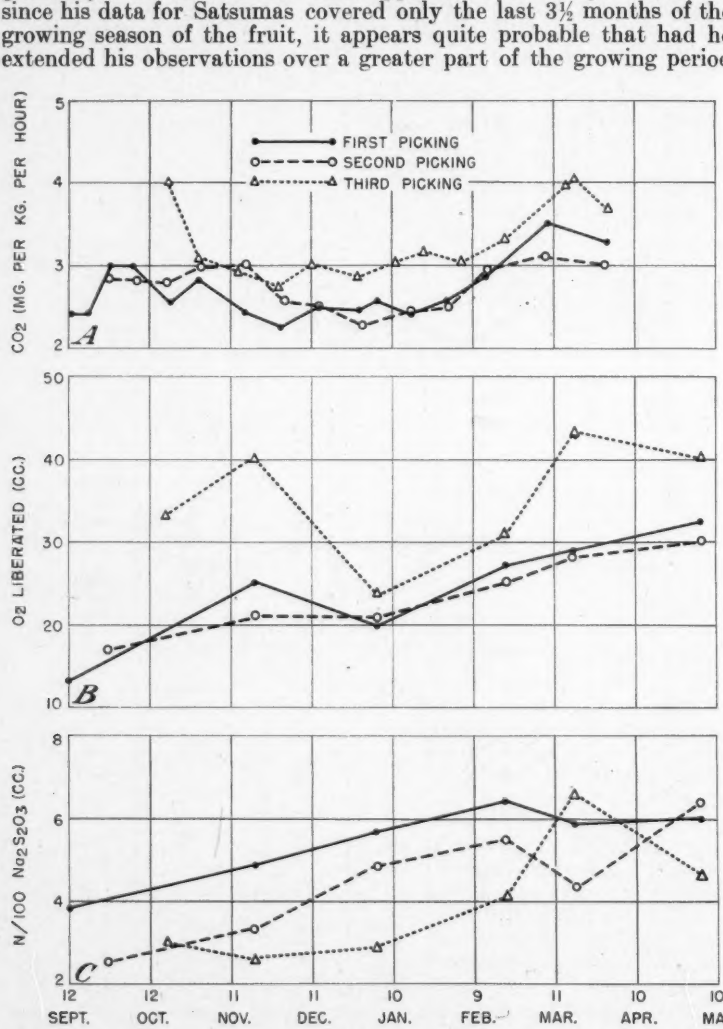

FIGURE 10.—Effect of maturity at harvest on rate of respiration (*A*), on catalase activity (*B*), and on oxidase activity (*C*) of Delicious apples during cold storage.

the curve obtained would also have been U-shaped. That this is true is suggested by the fact that the growth-rate curve of Satsuma oranges declines throughout the period covered. His data with the other fruits show that the growth rate begins to decline about the time the respiration begins to increase.

This change in rate of respiration of fruit during the harvest season and the subsequent effect in storage assume added interest when viewed from the storage standpoint. The rate of respiration determines the amount of heat generated by the fruit and must be taken into consideration in determining the amount of refrigeration necessary for properly storing or precooling the product. The results reported in this study show that the rate of respiration, and consequently the heat of respi-

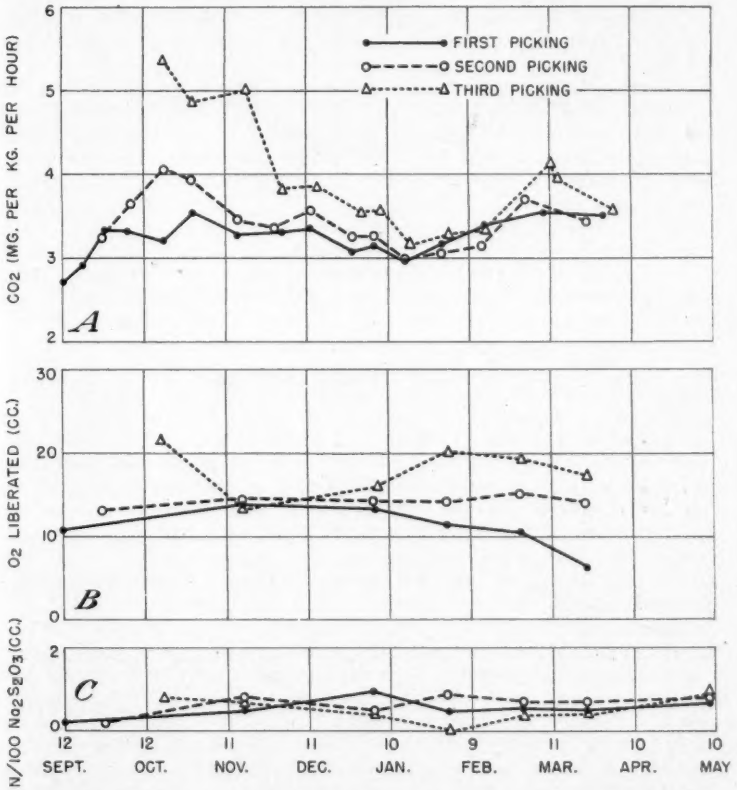

FIGURE 11.—Effect of maturity at harvest on rate of respiration (*A*), on catalase activity (*B*), and on oxidase activity (*C*) of Golden Delicious apples during cold storage.

ration, is not a stationary and definite figure for apples. Maturity, as well as the time elapsing between harvest and storage, exerts a considerable influence, and more refrigeration will be necessary for fully mature fruit than for fruit picked at a less mature stage.

In the present work the respiration measurements were made at a higher temperature than the minimum air temperature the night before harvest (fig. 1). Several workers have noted that when plant

tissues are moved from a lower to a higher temperature the rate of respiration increases to a point above that at which the tissues would normally respire at the higher temperature but that it later falls to the rate normal for the temperature. Willaman and Brown (*16*).

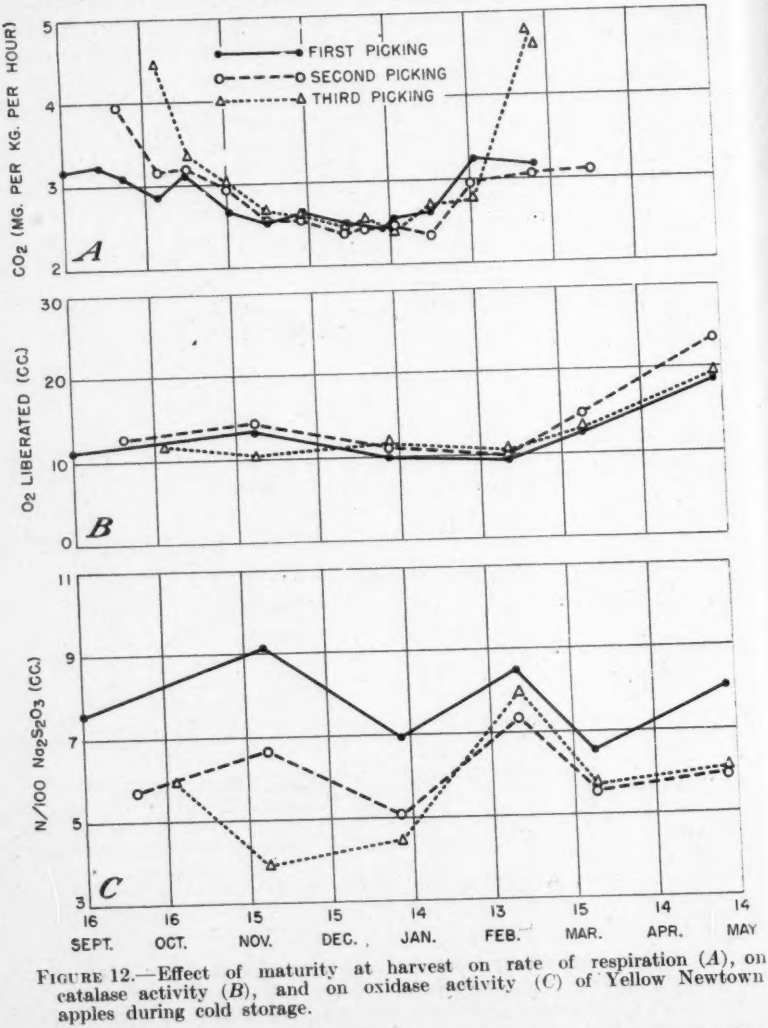

FIGURE 12.—Effect of maturity at harvest on rate of respiration (*A*), on catalase activity (*B*), and on oxidase activity (*C*) of Yellow Newtown apples during cold storage.

working with apple twigs, ascribed their results to a lower solubility of the carbon dioxide in the cell sap at the higher temperature, with a consequent release of the excess gas. Magness and Ballard (*13*) found no increase with Bartlett pears other than could be accounted for by the slightly riper condition of the cold-storage fruit. Harding

(*10*) studied the effect of alternating temperatures on the rate of respiration of Grimes Golden apples and concluded that "The rate * * * was neither stimulated nor depressed further (after the fruit samples had reached temperature equilibriums of 50° and 30° F.) than the respiration rate of fruit held constantly at the two temperatures." Magness and Burroughs (*14*) noted no difference with Winesap and Baldwin apples that could be ascribed to stimulation by low temperatures. However, Burroughs (*3*) reported some stimulatory effect on Wagener apples, especially in immaturely picked fruit. Kimbrough (*12*) reported a definite increase in respiration with potatoes, more excess carbon dioxide being given off than could be dissolved in the water of the potato at the storage temperature. He also determined the ratio of carbon dioxide to oxygen of the increased respiration and found it to be approximately 1, indicating that the excess was due to increased respiration rather than to dissolved carbon dioxide. Appleman and Smith (*2*) studied the effect of previous cold storage on a number of vegetables and found a definite stimulation with potatoes but no detectable increase with carrots. They concluded that the degree of stimulation varied with the vegetable and that those vegetables in which the percentage of starch was relatively high and in which there was a rapid shifting of the carbohydrate equilibrium with temperature changes were the ones that showed the greatest increase in initial respiration when they were transferred from a lower to a higher temperature. With potatoes Kimbrough (*12*) found that a storage period of about 3 weeks at the low temperature was necessary for the maximum stimulation when the tubers were moved to a higher temperature, and that the maximum respiration occurred on the second or third day after removal.

In the discussion of figure 1 (p. 456) it was pointed out that the minimum temperature the night before harvest was approximately 45° F. on August 8, September 8, and October 6, but that the respiration of Jonathan apples decreased in the period between the 5-hour and the 24-hour determination in the first two instances and increased in the last. This difference might indicate that maturity affects the time necessary for stimulation to take place. This conclusion, however, is opposed by the fact that the respiration of Yellow Newtown, a later maturing variety, increased in each instance. The divergence between the 5-hour and the 24-hour periods was greatest in the latter part of the season. Since this in general is the period when the lowest minimum temperatures were experienced, with a consequent greater increase from the minimum to the temperature at which the respiration was determined, perhaps a greater stimulation could be expected. From a review of the literature it appears extremely doubtful whether the fruit used in these experiments, if stimulated at all by change in temperature, was stimulated more than can be explained by the dissolved carbon dioxide in the cell sap. The results with potatoes where increased metabolic activity is probably connected with the transformation of sugar to starch may not be applicable to apples. At any rate it would be hard to explain satisfactorily the rise in respiration late in the season on the basis of stimulation due to increase in temperature.

The decrease in oxidase activity during the growing season is in agreement with the writers' results with Bartlett pears (*4*) and with

the results of Hinton (*11*) with apples. The catalase activity of apples increased during the growing period in contrast to that of pears, for which the values formed a U-shaped curve (*4*). If there is high catalase activity in apples when the fruits are very small, it drops off much earlier than it does in Bartlett pears, for there was no indication of it in these studies. The results obtained in the present work offer additional evidence that oxidase and catalase activity are not directly correlated with respiration or with each other (*5*).

Scald or soft scald of apples did not occur in the lots used in these experiments, so no direct conclusions can be drawn as to the relation of rate of respiration and enzyme activity at harvest to their development in storage. Since the respiration and enzymatic curves are so similar in varieties subject to soft scald and in those that are not, it appears doubtful whether the susceptibility of different varieties is due to differences in the rate of respiration or in oxidase or catalase activity at harvest.

SUMMARY

The effect of maturity on the rate of respiration and on the oxidase and the catalase activity of apples has been studied.

Respiration decreased during the early part of the growing season and reached a minimum prior to the commercial harvesting of the fruit. The time intervening between harvest and the measurement of the respired carbon dioxide may determine whether or not a later increase is shown.

Oxidase activity decreased during the growing season and may or may not increase if the fruit is left on the tree past the normal harvest season.

Catalase activity increased throughout the period from July to November.

Fruit harvested when fully mature usually showed a higher respiratory activity, a higher catalase activity, and a lower oxidase activity in storage than did fruit picked when less mature.

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FACTORS DETERMINING THE REDUCTION IN YIELD OF FIELD CORN BY THE EUROPEAN CORN BORER [1]

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INTRODUCTION

With the development of severe infestations of the European corn borer (*Pyrausta nubilalis* (Hbn.)) in the cornfields of Canada and the United States, questions arose regarding the reduction in yield resulting from definite populations of borers and also regarding the effect of plant development at the time of attack. The type and fertility of the soil were other factors that required consideration. All this information was needed as a guide in measuring crop losses and developing and interpreting control methods. It was also important to determine whether strains of corn (*Zea mays* L.) exist that will maintain their yields in the presence of a given population of borers to a greater degree than other strains of equal yielding capacity. Incidental to the study of these primary factors the data obtained provided an opportunity for studying the effect of the normal yield and the weather on borer damage.

The relation between type and fertility of the soil and yield of corn at definite borer populations was studied in northwestern Ohio with the variety Clarage from 1929 through 1933. The relation of plant development at the time of infestation and of the strain of corn to reduction in yield were studied primarily with hybrids and open-pollinated strains of corn at Sandusky and Toledo, Ohio, from 1930 through 1934.

METHODS

Several methods for determining the reduction in yield of plants containing different numbers of borers were tried. One method depended on obtaining the average yields of groups of plants containing the same number of borers in each plant and then comparing these yields with those produced by uninfested plants of the same strain. However, the moths' habit of laying more eggs on the taller, thriftier plants, which gave greater yields, and the higher rate of borer survival on such plants were complicating factors. Finally, the use of average yields and borer populations from plants in replicated plots was found to be the desired method. Different borer populations were induced by infesting the plants by hand with egg masses produced

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[2] The authors are indebted to members of the staff of the Ohio Agricultural Experiment Station for providing the seed of Clarage corn and a plot of ground at Holgate, Ohio, in 1932 and 1933. G. W. Conrey, J. S. Cutler, and J. B. McLaughlin, of the Station staff, assisted in the study by selecting the soil types, conferring with the authors during the progress of the work, and making equipment available for drying and shelling the samples. J. R. Holbert, formerly of the Bureau of Plant Industry, U. S. Department of Agriculture, furnished the seed of most of the hybrids. P. T. Ulman, of the Division of Entomology, Indiana Department of Conservation, provided a location for an experiment in 1933. The authors are indebted also to the following members of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture: D. J. Caffrey and W. A. Baker, for general supervision; and B. A. App, C. A. Crooks, and R. T. Everly, for assistance with the field work.

in the laboratory according to methods described by Patch and Peirce.[3] The eggs hatched within a day of their placement on the plants. The date of hatching was about 9 days later than the average date for eggs laid naturally. This delay is believed to have reduced borer damage, for it is shown later that the reduction in yield of plants becomes less as the stage of development at this time becomes more advanced.

Each replicated plot was divided into as many equal subplots as there were borer levels planned for in the experiment. Each subplot, except one left for natural infestation, was infested with a definite number of egg masses per plant, the number depending on the borer population desired. To keep newly hatched larvae from being windblown from higher to lower infestation levels, the infestations in the subplots decreased from left to right in the row of plots on the left side of the experimental field, but increased from left to right in the row of plots next to the right, and this alternation was repeated across the field. Prior to the migration of many full-fed borers in August, a sample of plants from each subplot was dissected to determine the mean number of borers per plant for each population level. The number and size of the replicated plots, the number of population levels, and the size of the plant samples varied with the experiment. In most of the tests with the Clarage variety 5 population levels were replicated 3 or more times, from 30 to 50 plants per population level were dissected to determine the mean borer populations, and about 300 plants per borer level remained for yield determination. In the tests with the hybrids samples of 150 plants were adequate for the determination of yields. The yields were calculated in bushels of shelled corn per acre on the basis of a moisture content of 15.5 percent.

RELATION BETWEEN YIELD OF CORN AND BORER POPULATION

In areas heavily infested with the borer, it appeared to some observers that a slight reduction in yield due to the borers occurred in fields infested with less than about 5 borers per plant and that increasingly greater damage was caused as the borer population increased above this level. To determine whether the regression of yield on borers was linear or curvilinear, the average yields for the hybrids for the years 1930, 1931, and 1932 were plotted against the different borer populations. Yields for Clarage were also plotted against borer populations, but in this case the results for each of 3 degrees of soil fertility were averaged for the entire period 1929–32. From a supplementary experiment with Clarage and Smoky Dent in 1931 and 1932, data for various population levels up to 22.5 borers per plant were available. Inspection of each year's data obtained from Clarage and Smoky Dent indicated a linear relationship, which justified their combination for graphical presentation in figure 1. In the case of the hybrids, however, the data from the individual hybrids for each year were not extensive enough to warrant a test of linearity, but a grouping of all hybrids for each year, as was done for figure 1, indicates a linear relationship. The conclusion may be made, therefore, that in the case of Clarage and Smoky Dent, and possibly in the case of the hybrids, the reduction in the yield of corn was proportional to the number of corn borers per plant.

[3] PATCH, L. H., and PEIRCE, L. L. LABORATORY PRODUCTION OF CLUSTERS OF EUROPEAN CORN BORER EGGS FOR USE IN HAND INFESTATION OF CORN. Jour. Econ. Ent. 26: 196–204, illus. 1933.

EFFECT OF NORMAL YIELD

While the effect of plant development at the time of borer attack and the effect of soil type and fertility on borer damage were being studied, it became apparent that direct comparisons were possible only when the estimated normal yields in the absence of borers were about the same. For this reason the effect of level of yield is discussed first.

The direct effect of yield level on the yield reduction per borer per plant was first observed on plantings of 24 hybrids at Sandusky and Toledo, Ohio. Five plantings were selected for study. Since in one planting the hybrids yielded from 57 to 105 bushels per acre and in

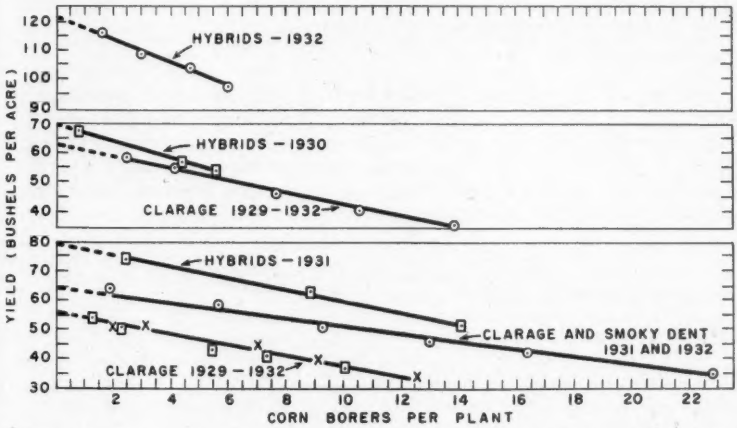

FIGURE 1.—Relationship between yield of corn and level of borer population induced experimentally. In the case of Clarage, 1929–32, plotted data from three levels of soil fertility are shown as follows: No fertilizer by squares, a single application of fertilizer by crosses, and a double application of fertilizer by circles.

another they yielded from 85 to 140 bushels, each planting included the range from 85 to 105 bushels. The average rate of yield reduction (B) in bushels per acre for each unit increase in borers and the estimated yield in the absence of borers (A) were calculated for each hybrid. In almost all cases the lowest population level was approximately 1 or 2 borers per plant; hence these estimates could be made with some confidence. The regression of B on A was then calculated for each planting (table 1). With the mean regression coefficient ($+0.078 \pm 0.0129$), the mean yield of the five plantings ($A=99.3$ bushels), and the mean yield reduction per borer per plant ($B=3.69$ bushels), the calculated yield reduction (B') was found to average 2.57 bushels when A was 85 bushels and 4.13 bushels when A was 105 bushels. Expressed in percentages, the rates of yield reduction were 3.02 and 3.93 percent when the normal yields were 85 and 105 bushels, respectively.

TABLE 1.—*Regression of yield reduction per unit of borer infestation (B) on the estimated yield of hybrid field corn in the absence of borers (A), Sandusky and Toledo, Ohio, 1931–34*

Planting date	Hybrids [1]	Average yield per acre in absence of borers (*A*)	Average per-acre yield reduction per borer per plant (*B*)	Regression of *B* on *A*
	Number	*Bushels*	*Bushels*	*Bushels*
May 25, 1931	15	82.0	2.97	+0.052±0.0197
May 13, 1932	22	120.8	4.05	+.070± .0136
May 26, 1933 [2]	11	99.3	3.30	+.058± .0205
May 17, 1934	17	96.6	3.61	+.095± .0413
May 31, 1934	18	97.9	4.53	+.115± .0384
Mean		99.3	3.69	+.078± .0129

[1] Hybrids having a statistically significant value for *B* were used.
[2] Since the mean values of *A* and *B* for the May 19 and June 2 plantings were nearly the same, data from both plantings were considered as one set.

Studies of the relation between level of yield and unit reduction in yield due to the borer under variable conditions of weather and soil fertility were made also on plantings of the Wooster strain of Clarage corn in several localities in northwestern Ohio from 1929 to 1933. The data for 18 of these plantings, including the estimated reduction in yield on the basis of regression, are given in table 2.

TABLE 2.—*Reduction in yield of shelled corn of the Clarage variety by the European corn borer, in various plantings in northwestern Ohio, 1929–33; plantings arranged in order of increasing yield*

Locality	Planting date	Estimated yield per acre in absence of borers (*A*)	Reduction in yield per acre per borer per plant (*B*)	Estimated reduction in yield per acre on basis of regression (*B'*)	Range in borer population per plant
		Bushels	*Bushels*	*Bushels*	*Numbers*
Sandusky [1]	May 13, 1929	28.2	1.6±0.35	1.37±0.204	1.2– 5.3
Holgate	May 26, 1933 [2]	40.8	1.7± .18	1.57± .137	.3– 6.6
Huron [1]	May 15, 1929	42.4	1.2± .25	1.59± .130	1.6– 9.2
Toledo	May 10, 1932	42.4	1.5± .10	1.59± .130	1.6– 8.0
Huron [1]	May 9, 1930	43.5	1.8± .14	1.61± .126	1.1– 5.8
	May 7, 1931	43.7	2.0± .02	1.62± .125	2.3–13.7
Maumee	May 11, 1932	45.5	1.6± .22	1.64± .118	1.3–10.1
	May 26, 1933 [2 3]	48.1	4.4± .29	1.69± .119	.6– 4.4
Toledo	May 17, 1929	50.4	1.0± .04	1.72± .105	5.8–32.1
	May 13, 1931 [1]	52.5	1.7± .16	1.76± .103	.4– 9.9
Sandusky [1]	May 7, 1930	53.8	2.5± .19	1.78± .102	1.3– 6.1
Toledo	May 6, 1931	55.0	1.0± .14	1.80± .102	1.0–27.3
Holgate	May —, 1932	56.8	2.1± .47	1.83± .103	1.4– 8.0
Toledo	May 11, 1932	59.4	1.8± .19	1.87± .107	2.4–11.4
Sandusky [1]	May 6, 1931	66.5	1.9± .08	1.98± .130	2.6–19.3
Toledo	May 26, 1933 [2]	80.4	2.8± .35	2.20± .201	3.2–14.4
	May 5, 1932	80.8	1.9± .24	2.21± .204	2.0–22.7
Sandusky [1]	May 17, 1932	84.6	2.3± .22	2.27± .226	2.7– 8.9
Mean	May 13	54.5	1.79		1.8–12.4

[1] Average of 3 levels of soil fertility.
[2] Average of 2 plantings made on May 19 and June 2.
[3] Not included in calculating the regression coefficient.

From the values in table 2 the regression of *B* on *A* was calculated. With 15 degrees of freedom the regression coefficient, 0.016 ± 0.0067 bushel, may be regarded as significant. With this regression coefficient, the mean yield of the 17 plantings ($A = 54.5$ bushels), and the mean yield reduction per borer per plant ($B = 1.79$ bushels), it was calculated that B' would average 1.37 bushels when A was 28.2

bushels and 2.27 bushels when A was 84.6 bushels. Expressed in percentages the rates of yield reduction were 4.86 and 2.68 percent when the normal yields were 28.2 and 84.6 bushels, respectively. The estimated reduction in yield on the basis of regression was also calculated for each planting of Clarage listed in table 2.

Before comparisons are made between the data from Clarage and from the hybrids, the conditions under which the two sets of data were obtained should be examined. Since the reduction in yield due to the borer increases as the stage of plant development at the time of borer hatch becomes less advanced, the question arises whether the greater unit reduction in yield for the hybrids at the highest level was directly associated with increase in the level of yield or was caused by greater damage to later silking hybrids that yielded more. In the five plantings under consideration (table 1), on an average 82.6 percent of the hybrids silked within 3 days, and on one planting in each of 3 years 13, 14, and 11 hybrids, respectively, silked within 2 days. For hybrids having the same or the succeeding day as their average silking date, it was determined that for each planting the regression of B on A was significant. As an average of these 3 plantings, for each unit increase in the normal yield the yield reduction per borer per plant increased 0.072 ± 0.0154 bushel as compared with 0.078 bushel, the average value for the five plantings. The generally narrow range in silking dates and the highly significant regression of B on A obtained for the hybrids silking within 2 days indicate that greater damage per borer at higher levels of yield was directly associated with the increase in the level of yield in the plantings considered.

In the case of Clarage there were only slight differences in plant development at time of infestation between the plantings giving the largest and the smallest yields. No reason is known why the greater unit reduction in yield at the high yield level may not be considered as being also directly associated with increase in the level of yield.

From a study of the data from Clarage and the hybrids the following comparisons are noteworthy:

(1) On the basis of a purely mathematical relationship a constant percentage of the crop would be expected at all yield levels. In Clarage, however, the loss was found to be 4.86 percent of the yield per borer per plant at the yield level of 28.2 bushels and 2.68 percent at the yield level of 84.6 bushels. Evidently there were factors involved to change the expected relationship. It is possible that the uninjured tissue within the stalk is progressively able to do more of the work of the injured tissue as the plant's environment enables it to yield more. As a matter of fact, with hybrids a given number of borers were found to reduce the yield of plants in the same field only about half as much in the relatively wet season of 1931 as in the 1930 season of drought.

(2) In Clarage the percentage reduction in yield was considerably greater at the lowest level of yield than at the highest level, whereas in the hybrids the percentage reduction was greater at the highest level of yield. The reason for this reversal in trend is not known.

(3) At the yield level of 85 bushels, B' was calculated to be 3.02 ± 0.249 percent per borer per plant for the hybrids and 2.68 ± 0.268 percent for Clarage. The difference is not significant. It is possible, however, that some of the difference in favor of Clarage may have

492552—42——4

been due to the difference in planting date, which averaged 9 days earlier for Clarage. At the time of infestation Clarage was in a more advanced stage of development than the hybrids. Another study [4] showed that hybrids were more tolerant to the corn borer than open-pollinated varieties in the same plantings, the percentage of yield reduction being less for the hybrids. In the present study it is impossible to compare the hybrids and open-pollinated Clarage for relative tolerance.

EFFECT OF WEATHER

In the planting of Clarage made on May 7, 1930, at Sandusky (table 2), the difference between the observed and the predicted rate of yield reduction may be regarded as highly significant. The drought of 1930 is suggested as an explanation for the high observed rate. The rainfall from 6 showers during the critical period of growth, from June 20 to August 20, totaled only 1.83 inches with a maximum of 0.60 inch, and the temperature averaged 2.2°, 1.8°, and 1° F. above normal for June, July, and August, respectively. Moreover, in another field on the same farm 28 hybrids and open-pollinated varieties also showed a high yield reduction per borer per plant. The hybrids and open-pollinated varieties were planted a day earlier than Clarage. Their estimated average normal yield of 69.3 bushels was reduced on an average 2.9 ± 0.11 bushels per borer per plant as compared with an average reduction of 1.5 ± 0.14 bushels from 14 strains planted in the same field on May 5 and 12, 1931, on which the normal yield averaged 70 bushels per acre. From June 20 to August 20, 1931, 18 showers of more than 0.04 inch rainfall totaled 7.60 inches with maxima of 0.65 0.35, and 1.68 inches in June, July, and August, respectively. The temperature averaged 1.0°, 4.6°, and 2.1° above normal in the 3 months. The corn-growing season of 1931 was therefore more favorable than that of 1930, for only about half as much yield reduction per borer occurred in strains planted in the same field and having about the same level of yield.

The rates of reduction in yield due to the borer for the 1931 planting at Huron and especially for the 1933 planting at Maumee were also significantly greater than those predicted on the basis of normal yield (table 2). A planting in 1932 at the Maumee location showed little difference between observed and estimated reduction in yield. The level of yield was the same, but the field was planted earlier than in 1933. Although weather records were not taken at the Maumee location, the 1933 season was in general drier than the 1932 season. Dry conditions may have contributed to the high rate of borer damage on this planting as well as at Sandusky in 1930.

EFFECT OF SOIL FERTILITY

In eight of the plantings of Clarage the experiment was conducted at three levels of soil fertility—(1) on soil receiving no fertilizer, (2) on soil receiving a unit application of fertilizer, and (3) on soil receiving twice as much as (2). The fertilizer varied in amount and formula with the soil type. The differences in yield reduction per borer per plant between the three fertility levels were not significant

[4] PATCH, L. H., STILL, G. W., AFF, B. A., and CROOKS, C. A. COMPARATIVE INJURY BY THE EUROPEAN CORN BORER TO OPEN-POLLINATED AND HYBRID FIELD CORN. Jour. Agr. Res. 63: 355-368. 1941.

in most of the plantings. When averaged for the eight plantings, the estimated yield of 48.3 bushels per acre in the absence of borers was reduced 2.0 ± 0.17 bushels per borer per plant where no fertilizer was applied, the yield of 50.7 bushels was reduced 1.7 ± 0.10 bushels where the unit amount of fertilizer was applied, and the yield of 56.8 bushels was reduced 1.9 ± 0.09 bushels where twice the amount of fertilizer was applied. Since the differences in yield reduction are not statistically significant, and the differences among the normal yields are not large, it appears that the fertility of the soil had little effect, if any, on the damage by the corn borer in these tests.

EFFECT OF TYPE OF SOIL

In the plantings listed in table 2 the type of soil also varied. At Huron the field was a clay loam derived from shale-sandstone, at Sandusky it was a very fine sandy loam, at one location in Toledo the soil was a very fine sandy loam and at another silty clay, at Maumee the soil was light-colored Fulton silt loam, and at Holgate it was black Brockston clay. On the very fine sandy loam at Sandusky in 1929, 1930, and 1931 the average estimated yield of Clarage in the absence of borers was about 49.7 bushels per acre; the corn borer reduced this yield at the average rate of 2.0 ± 0.10 bushels per borer per plant. On the clay loam from shale-sandstone at Huron the average yield of 43.8 bushels for the same years was reduced at the rate of 1.7 ± 0.11 bushels per borer per plant. The difference between the rates of yield reduction on these two types of soil is only what might be expected if the rate of reduction is considered a function of the level of normal yield. Other comparisons between types of soil furnished no evidence that any factors other than level of yield are involved.

EFFECT OF STAGE OF PLANT DEVELOPMENT

In studies on the effect of stage of plant development at the time of borer hatch on yield reduction due to the corn borer, the interval between the hatching of the borers and the silking of the corn was varied (1) by infesting samples of plants of the same planting on different dates and (2) by infesting samples of different plantings on the same date.

In 1932 an experiment was conducted to determine the rate of yield reduction of plants infested at different times. Two open-pollinated varieties and four hybrids were planted on May 16 in 8-hill plots replicated 15 times. Four borer levels were used, the plants were infested on 3 dates, and the yield was based on a total of about 160 plants from the 6 strains of corn for each borer level of plants infested on each date. The average date of silking was July 26, and in the absence of borers the estimated yield should have averaged 84.1 bushels per acre. This potential yield was reduced 3.5 ± 0.85 bushels per borer per plant when the plants were infested on June 27, 2.8 ± 0.53 bushels when infested on July 8, and 1.9 ± 0.31 bushels when infested on July 18.

Although the earliest infested plots showed the greatest reduction in yield, the differences between the rates were not found to be significant. It is probable, however, that larger samples of plants,

or more borer levels, would have reduced the variability of the data sufficiently to show significance, since data derived from other sources show a significant trend of change in the rate of reduction of yield associated with stage of plant development.

The rates of yield reduction in various plantings in which the infestation date for the plantings within a season was uniform are given in table 3. Since the mean levels of yield for the earliest and latest plantings differ by less than a bushel, the rates of yield reduction are directly comparable. The difference of 1.6 ± 0.30 bushels may be regarded as highly significant with the 156 degrees of freedom available. This difference is associated with the difference of 23 days between the average dates of the earliest and latest plantings, and an interval of 11 days from egg hatching to corn silking. The yield reduction per borer was 2.85 percent of the normal yield of the earliest plantings as compared with 4.71 percent for plantings made 23 days later. An average of five borers per plant in each of the earliest plantings would have reduced the yield nearly as much as an average of three borers per plant in each of the latest plantings, because a larger percentage of the late-planted crops was destroyed by the borer. It is probable that the plants of the late plantings infested early in their development were subject to greater reductions in yield, owing to the longer duration of borer feeding before the critical period of ear production and the consequent weaker condition of the plants and the larger average size of the borers during the period of ear production.

TABLE 3.—*Reduction in yield of shelled corn by the European corn borer, Ohio and Indiana, 1931–34*

Locality	Date of planting	Mean date of silking	Period from egg hatching to corn silking	Estimated yield per acre in absence of borers	Observed reduction in yield per acre per borer per plant	Degrees of freedom
	1931		*Days*	*Bushels*	*Bushels*	
Sandusky, Ohio	May 5	July 27	11	84.8	1.6±0.10	24
	May 12	July 28	12	80.2	1.7±.10	24
	May 19	July 31	15	74.9	2.2±.21	24
	May 25	Aug. 1	16	76.5	2.5±.21	24
	1932					
	May 7	July 14	9	94.0	2.9±.46	11
	June 7	Aug. 2	26	96.7	6.1±1.00	11
	1933					
Toledo, Maumee, and Holgate, Ohio, and Auburn, Ind.[1]	May 19	July 30	16	67.1	2.7±.10	19
	June 2	Aug. 5	22	71.9	3.0±.22	19
	1934					
Toledo, Ohio	May 3	July 17	9	91.1	2.3±.12	24
	May 17	July 25	17	91.4	3.3±.17	24
	May 31	Aug. 2	25	94.9	4.3±.19	24
Mean for period 1931–34:						
Earliest planting	May 9	July 22	11	84.3	2.4±.12	11
Latest planting	June 1	Aug. 3	22	85.0	4.0±.27	22

[1] Data are averages from the 4 localities.

EFFECT OF STRAIN OF CORN

The data from the hybrids obtained over the period 1930–34 were used to test the significance of the deviation of each hybrid from the regression line of B on A. The basic data were the same as those used for calculating the data in table 1. The observed rate of yield reduc-

tion, B, was found to be significantly less than the rate predicted on the basis of regression on A in only 4 out of 149 tests, 2 of which were of the single-cross hybrid Ill. A × Ind. TR. Apparently few hybrids are able to maintain their yield in the presence of a given number of borers to a greater degree than other hybrids of equal yielding capacity.

The yield of hybrid Ill. A × Ind. TR should have been reduced 3.67 bushels per acre for each additional borer per plant had it reacted to the borer according to prediction. Actually its yield was reduced on an average 2.35 bushels per borer per plant. Patch and others [5] [6] have found, however, that the survival of borers on this hybrid is higher than the average. Hence, any advantage from having smaller losses in yield per borer is partly, if not wholly, offset by the greater than average number of borers to cause reductions in yields.

In 1931 and 1932 a small-stalked early variety, Smoky Dent, and a medium-stalked midseason variety, Clarage, were compared with each other and with a large-stalked late variety, Johnson County White. The interval from planting to silking averaged 71, 78, and 89 days, and the height of the plants at maturity averaged 90, 103, and 108 inches, respectively. The peak of hand infestation was 15 days later than the peak of natural infestation in 1931 and 14 days later in 1932. In another experiment with the same varieties in 1933 the diameter of the plants averaged 0.81, 0.83, and 1.02 inches at the second internode.

As an average of the tests of 1931 and 1932, the normal yield of Smoky Dent of 63.6 bushels was reduced 1.3 ± 0.06 bushels per borer per plant, the yield of Clarage of 67.9 bushels was reduced 1.5 ± 0.14 bushels, and the yield of Johnson County White of 79.1 bushels was reduced 2.2 ± 0.21 bushels. These rates of yield reduction are lower than the rates expected for the yield levels indicated, probably because the plants were infested later than usual. The small difference in the rate of yield reduction between Smoky Dent and Clarage is not significant. The difference of 4.3 bushels between the normal yields of the two strains is also small. A greater reduction in yield that possibly might have been expected on account of the smallness of Smoky Dent might well have been offset by the decrease due to its earliness, for the interval between borer hatch and the beginning of ear development would have been shortened, and consequently its plants were probably in a less weakened condition than were those of Clarage during the period of ear development.

Johnson County White had an average yield of 11.2 more bushels per acre than Clarage, and it silked 11 days later. Its yield was reduced at a significantly greater rate than the yield of Clarage. A decreased rate of yield reduction that possibly might have been expected on account of the large size of Johnson County White might well have been offset by the increase due to its lateness and higher level of yield. Because of its lateness Johnson County White would have been in a more vulnerable condition than Clarage during the period of ear development.

Neiswander and Herr [7] also studied the reduction in yield of early and later maturing strains infested by the corn borer. Using Burr

[5] PATCH, L. H. RESISTANCE OF A SINGLE-CROSS HYBRID STRAIN OF FIELD CORN TO EUROPEAN CORN BORER. Jour. Econ. Ent. 30: 271–278. 1937.

[6] PATCH, L. H., BOTTGER, G. T., and APP, B. A. COMPARATIVE RESISTANCE TO THE EUROPEAN CORN BORER OF TWO HYBRID STRAINS OF FIELD CORN AT TOLEDO, OHIO. Jour. Econ. Ent. 31: 337–340. 1938.

[7] NEISWANDER, C. R., and HERR, E. A. CORRELATION OF CORN BORER POPULATION WITH DEGREE OF DAMAGE. Jour. Econ. Ent. 23: 938–945, illus. 1930.

Leaming and Smoky Dent, silking 6.7 days earlier than Burr Leaming, as two field-corn varieties, and Golden Bantam, a sweet-corn variety silking 4.1 days earlier than Smoky Dent, they concluded that their data indicated "considerable variation in the damage resulting to different varieties from a given borer population, the amount of damage per borer increasing as the length of growing season of the variety decreases." Comparisons were made on the basis of yields taken after the ears of the field-corn varieties were mature and while Golden Bantam was in the roasting-ear stage. In the present study of field corn no evidence was found to support their conclusion. Other studies indicate that field- and sweet-corn varieties are not directly comparable as to damage by the corn borer.

SUMMARY

The degree of reduction in yield of field corn resulting from definite levels of population of the European corn borer was determined by manually infesting plants with various numbers of egg masses. Reduction in yield within cornfields is shown to be proportional to the number of borers present up to 22 borers per plant. Within the range from 28 to 85 bushels per acre the fields that would have produced greater normal yields in the absence of borers had smaller percentages of their crops destroyed by a given number of borers than the fields with lower yields. Data from plantings of the Clarage variety in various localities in northwestern Ohio from 1929 to 1933 showed that the rates of yield reduction were 2.68 and 4.86 percent per borer per plant when the normal yields were 85 and 28 bushels per acre, respectively. The rate of yield reduction for the hybrids was 3.93 percent per borer per plant when the normal yield was 105 bushels per acre as compared with 3.02 percent when the normal yield was 85 bushels.

The rate of yield reduction per borer in corn planted on soils differing in type or fertility is probably a direct function of the level of normal yield. In seasons of drought the damage per borer at given yield levels appears to be increased.

Stage of plant development at time of infestation is shown to be an important factor in yield reduction in the presence of the borer. Plants infested early in their development suffered greater rates of yield reduction, owing to the longer duration of borer feeding before the critical period of ear production and the consequent weaker condition of the plants, and to the larger average size of the borers during the period of ear production. The average normal yield of 85 bushels per acre over a 4-year period, for corn planted on the average date May 9, was reduced 2.85 percent per borer as compared with 4.71 percent for the same hybrids giving about the same average yield but planted 23 days later. An average of five borers per plant in each of the earliest plantings, therefore, would have reduced the yield nearly as much as an average of three borers per plant in each of the latest plantings.

Attempts were made, without much success, to find hybrids having the ability to maintain their yields in the presence of a given level of borer population to a greater degree than would other hybrids of equal yielding ability.

HYDROCEPHALUS, A LETHAL IN CATTLE[1]

By C. L. COLE, *research associate in animal husbandry*, and L. A. MOORE, formerly *research assistant in dairy husbandry, Michigan Agricultural Experiment Station*

INTRODUCTION

Hydrocephalus has been reported as an inherited lethal in swine (*1*)[2] and mice (*2*, *4*). Many sporadic cases have been reported in man, cattle, chickens, dogs, and rats. There are two types variously reported: (1) Internal hydrocephalus, a collection of fluid in the cerebral ventricles, and (2) external hydrocephalus, a collection of fluid outside the brain substance.

Houck (*7*) reported two cases of congenital hydrocephalus, one a grade Durham calf and the other an albino rat. The condition was not lethal in either instance. In the rat, however, blindness was associated with hydrocephalus and the animal eventually died from starvation.

Clark (*2*) has noted hereditary hydrocephalus in the house mouse which appeared in the F_2 and backcross generations of mice with the flexed-tail character. It was apparently due to a single Mendelian factor, the homozygous recessive being lethal. Frequent association with the flexed-tail character suggested linkage or another manifestation of the gene for flexed (*2*). Later work (*3*) showed that hydrocephalus was not caused by the same gene as flexed tail. Still further experiments (*4*) showed no linkage between hydrocephalus and various other characters, including flexed tail.

Blunn and Hughes (*1*) have recorded a lethal hydrocephalus of the external type in Duroc-Jersey swine, the fluid being found outside the brain in the subarachnoid spaces. Varying degrees of hydrocephalus occurred, but a short tail or no tail always accompanied the defect regardless of its degree. Light coat color also accompanied the defect in all except 3 cases. All the affected pigs were the result of inbred matings. Twenty litters from heterozygous parents produced 178 animals, of which 42 were hydrocephalic and 136 normal. It was suggested that the three associated characters might be the manifold effects of one factor, or they might be caused by very closely linked genes. The goodness of fit between the observed and expected 3 : 1 ratio (χ^2=0.187. P=between 0.5 and 0.7) indicates that the hydrocephalus syndrome was caused by a single recessive autosomal gene.

Hyde (*8*) observed an epidemic of hydrocephalus in experimental rabbits, but was unable to offer any rational explanation for its occurrence or disappearance.

Ely, Hull, and Morrison (*6*) reported one case of slight hydrocephalus among four cases of agnathia in Jersey calves.

Morrill (*10*) observed that hydrocephalus occurs in certain breeds of dogs, notably in the German boxer, and he recorded occasional

[1] Received for publication February 5, 1942.
[2] Italic numbers in parentheses refer to Literature Cited, p. 490.

observations in single individuals of other breeds. He studied several purebreds and various crosses. Among the purebreds, the British bull showed marked external hydrocephalus in four out of five cases examined; one case was practically normal. Among hybrids, the condition was most often seen in the great Dane × St. Bernard crosses, but in varying degrees.

Schlotthauer (*11*) noted five random cases of internal hydrocephalus in dogs. In only one case was there any evidence that the condition was hereditary.

Eaton (*5*) summarized 15 known lethals in cattle. None of these corresponds in any way with the lethal reported here.

EXPERIMENTAL RESULTS AND OBSERVATIONS

The lethal to be described was discovered in a herd of grade and purebred Holstein-Friesian cattle. Purebred bulls had been used for several generations, but insofar as could be determined, no inbreeding had been practiced before 1938. Early in 1940 the owner interviewed one of the authors relative to a possible nutritional deficiency in his herd. In December 1939, an abnormal calf had been born but no particular significance was attached to the occurrence. However, when another calf with the same abnormalities was born in January 1940, the owner became worried about his ration. A careful check revealed that the herd had been fed legume roughage, mainly alfalfa hay with some sweetclover, and a grain mixture composed of corn and cob meal, oats, and soybean oil meal. The cattle were given free access to a mixture of 1 part bonemeal and 2 parts salt during the winter months, and salt alone during the pasture season. There seemed to be no reason for suspecting a nutritional deficiency and the two cases suggested something other than chance. A check on the breeding program revealed that the two heifers that had given birth to the abnormal calves had been mated to their sire. Previous to these two abnormalities, no unusual calves had been born except that in June 1939 a calf from a similar mating had shown a highly nervous condition, and was unable to stand alone. No improvement of this condition was observed and the calf was later vealed. In December 1939 and January 1940 two calves were born from sire-daughter matings that were normal in every way, except for asymetrical faces. A similar "wry jaw" defect was reported by Lutikov (*9*) in a study of breeding methods practiced in a herd of Jaroslav cattle.

The breeding program in this herd suggested an inherited condition and so arrangement was made with the owner whereby an additional number of sire-daughter matings would be made. Twenty-seven calves were produced as a result of sire-daughter matings, involving 15 daughters. The results of these matings are given in table 1.

TABLE 1.—*Date of calving, and condition and sex of calf from sire-daughter matings involving 15 daughters*

Cow No.	Date of calving	Condition of calf	Sex
23	Nov. 24, 1938	Normal	Male.
	Jan. 23, 1940	Lethal	Do.
	Dec. 30, 1940	do	Do.
24	June 1, 1939	Jumpy	Female.
	May 8, 1940	Normal	Do.
	Apr. 21, 1941	do	Male.

TABLE 1.—*Date of calving, and condition and sex of calf from sire-daughter matings involving 15 daughters*—Continued

Cow No.	Date of calving	Condition of calf	Sex
25	June 26, 1939	Normal	Male.
	May 16, 1940	do	Female.
27	Dec. 6, 1939	do	Male.
	Nov. 3, 1940	do	Female.
28	Dec. 25, 1939	Lethal	Male.
	Mar. 1, 1940 [1]	Normal fetus	Undetermined
29	Dec. 27, 1939	Asymmetrical	Female.
	Jan. 31, 1941	Lethal	Male.
30	Dec. 27, 1939	Normal	Do.
	Jan. 1, 1941	Lethal	Do.
31	Jan. 12, 1940	Asymmetrical	Female.
	([2])	Normal fetus	Undetermined.
32	Feb. 4, 1940	Normal	Male.
	Jan. 15, 1941	do	Do.
33	Mar. 22, 1940	do	Female.
	Mar. 16, 1941	do	Do.
35	Dec. 22, 1940	do	Male.
36	Jan. 7, 1941	do	Female.
37	Feb. 7, 1941	Jumpy	Do.
39	May 21, 1941	Asymmetrical and lethal	Do.
40	June 12, 1941	Normal	Male.
Totals (15 cows, 27 calves)		5 lethal 1 asymmetrical and lethal 2 asymmetrical 2 jumpy 17 normal	14 males. 11 females. 2 undetermined.

[1] Cow butchered in advanced pregnancy.
[2] Cow died in advanced pregnancy.

The lethal was manifested by an internal hydrocephalus accompanied by a marked papilledema. The lateral ventricles were greatly distended so that only a thin layer of cerebral tissue remained between the cavity of the ventricles and the cranial bones. On dissection, no abnormalities were found which might suggest a lack of embryological development of the arachnoid villi, nor were there any apparent points of blockage in the various foramina so far as could be determined. As a result of the pressure developed, the cranial cavity of the skull was enlarged from two to three times the normal size, as shown in figures 1 and 2.

Ventrally the position and angle of the foramen magnum was markedly altered from that of a normal animal, as shown in figure 3. It is possible that this abnormality might partly or completely block the foramina of Magendie and Luschka, thereby causing the hydrocephalus as a secondary manifestation of a gene for bony abnormalities. Figure 3 shows also a marked widening of the space between the mandibles as compared to the normal.

Both the humeri and femurs of these animals showed marked malformation. The shaft of these bones was considerably shortened, but larger in diameter than the normal, as shown in figure 4.

The condyles and heads of both bones had the appearance of having had pressure applied against the articulating surfaces so that they were bent down toward the shaft and twisted to one side. Previous to dissection, the twisted condition of the femur resulted in extreme width through the hips, causing difficult parturition. The abnormal humerus also resulted in a twisted appearance of the forelegs (fig. 5).

FIGURE 1.—Frontal view of hydrocephalus calf, showing marked enlargement of the skull; note twisted condition of forelegs.

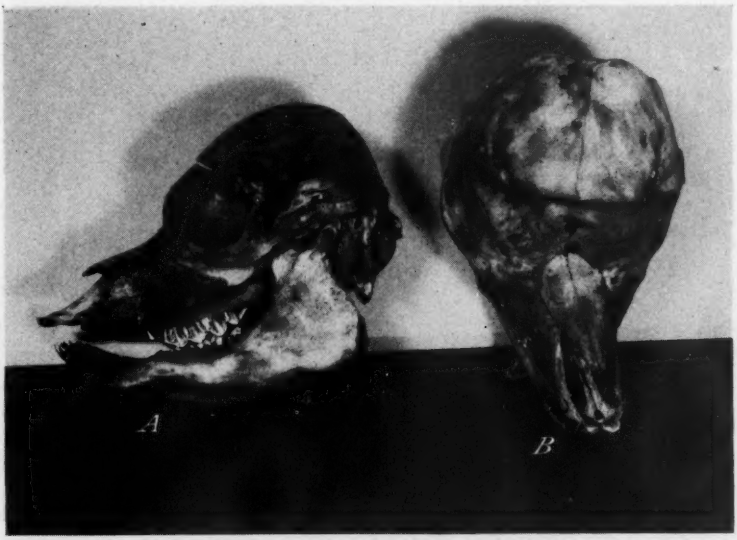

FIGURE 2.—Side view (*A*) and frontal view (*B*) of hydrocephalus skulls; *B* shows asymmetrical condition.

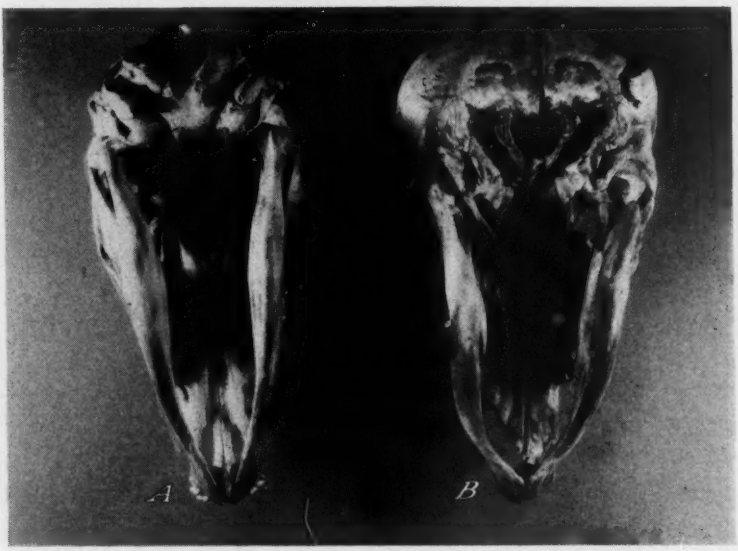

FIGURE 3.—View of skulls showing position of foramen magnum and also abnormal condition of mandibles: *A*, normal; *B*, lethal.

FIGURE 4.—Humerus and femur from an abnormal (*A*) and a normal calf (*B*).

FIGURE 5.—View of abnormal calf showing extreme width of hips and twisted forelegs.

The asymmetry of the skull and face was shown most distinctly in the living animal by a twisting of the face to one side. When the head was cleaned of surrounding tissues, asymmetry of the lower jaw and facial bones was quite apparent. In addition, the bony structures of the cranium were unequally developed and displaced to one side of the midline. In the specimen photographed in figure 2, which is typical of these individuals, the unequal development of the cranial bones is well shown, as is the twisted appearance of the face. In addition, the left mandible was considerably shorter than the right. This was also true for the maxillae.

The condition designated as "jumpy" was suggestive of cerebellar or thalamic dysfunction. Marked lack of muscular coordination and control was at all times evident. These calves were unable to rise or stand unaided. When placed on their feet, the weight was supported reasonably well for a short time at least. In this position, however, the animal tended to sway from side to side or backward and forward and unless supported was unable to remain standing. Tremor was present in both the upright and lying positions. In the lying position, in addition to the tremor, there was almost constant movement of the limbs. Resistance to flexion of the limbs was present irregularly.

Possibly the lethal trait, the asymmetry, and "jumpy" are due to three recessive autosomal genes.

A genetic analysis based on the above hypothesis may now be attempted. The three pairs of allels would be as follows:

L—normal	*l*—lethal
A—symmetrical	*a*—asymmetrical
J—normal nervous reaction	*j*—"jumpy"

The sire should have the genotype *L1AaJj*.

The daughters from normal dams would have eight different genotypes, as follows: *LLAAJJ*, *LLAAJj*, *LLAaJJ*, *LLAaJj*, *LlAAJJ*, *LlAaJJ*, *LlAAJj*, *and LlAaJj*. These daughters backcrossed to their sire would then produce the following percentages of phenotypes in their offspring: *LAJ*, 66.992; *LAj*, 9.570; *LaJ*, 9.570; *lAJ*, 9.570; *lAj*, 1.367; *laJ*, 1.367; *Laj*, 1.367; and *laj*, 0.195.

The expected and observed distribution of the 27 calves by phenotype would, therefore, be as shown in table 2. In this table *lAJ* and *lAj* are combined as are also *laJ* and *laj*. These combinations must be made because of the impossibility of observing the "jumpy" condition in the "*l*" calves which are born dead. The goodness of fit between the observed and expected values ($\chi^2 = 2.92$; P = between 0.7 and 0.8) suggests the action of three recessive nonlinked genes.

TABLE 2.—*Expected and observed distribution of the 27 calves by phenotype*

Distribution	*LAJ*	*LAj*	*LaJ*	*lAJ—lAj*	*Laj*	*laJ—laj*
Expected	18.09	2.58	2.58	2.95	3.37	0.42
Observed	17.0	2.0	2.0	5.	0	1.0
Difference	−1.09	−.58	−.58	+2.05	−.37	+.58

Breaking the trihybrid ratio down into three monohybrid ratios, the expected and observed values are as shown in table 3, which also shows the expected and observed incidence of the "jumpy" animals among the nonlethals.

TABLE 3.—*Expected and observed values for lethal-normal, asymmetrical-normal, and "jumpy"-normal animals*

Contrasted types	Ratio—		χ^2 [1]	*P*
	Expected	Observed		
Lethal-normal	3.38 : 23.62	6 : 21	2.32	Between 0.2 and −0.1.
Asymmetrical-normal	3.38 : 23.62	3 : 24	.05	Between 0.9 and −0.8.
"Jumpy"-normal	2.63 : 18.37	2 : 19	.17	Between 0.7 and −0.5.

[1] Although the chi-square test has been used, the numbers are smaller than are desirable for such an analysis.

CONCLUSIONS

A new lethal in cattle, internal hydrocephalus, is described and shown to be probably a simple recessive in its mode of inheritance.

Two other conditions, asymmetry and "jumpy," are described and may be recessive and not linked with each other or with the lethal gene.

The data suggest that the sire used carried three rare recessive genes. Nevertheless, the probability that any one animal would carry three rare factors is very small. However, the facts are stated and a conclusion indicated, though the mode of inheritance of the characters "jumpy" and asymmetry should be studied further.

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DEVELOPMENT OF SYNTHETIC FOOD MEDIA FOR USE IN NUTRITION STUDIES OF THE EUROPEAN CORN BORER [1]

By G. T. BOTTGER

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INTRODUCTION

Studies on the nutrition of the European corn borer (*Pyrausta nubilalis* (Hbn.)), since their initiation in 1937 at the Toledo, Ohio, research laboratory, have been directed principally toward finding chemical substances contained in green plant tissue, particularly corn (*Zea mays* L.), which are associated with the survival, growth, and metamorphosis of the insect. Preliminary studies reported by Bottger [3] and subsequent feeding tests, supplemented with chemical analyses of the test materials, besides adding appreciably to available information relative both to the insect and to the chemistry of various strains of corn, have emphasized a long-recognized need for a synthetic food medium that would facilitate a more comprehensive study of the nutritive requirements of the organism. Such a medium would eliminate the necessity of difficult, and often unsatisfactory, chemical analyses and would permit an accurate comparison of the effect of each nutritive constituent, including the vitamins, on the physiological development of the insect and its relation to borer-resistant strains of corn.

On the basis of information accumulated from various experiments, a series of tests was designed for the purpose of rearing corn borer larvae to maturity on a synthetic medium.

METHODS, TEST MATERIAL, AND EQUIPMENT

Twenty synthetic food media were formulated to simulate roughly in carbohydrate, protein, and moisture content some samples of green corn tissue obtained through the cooperation of the Bureau of Plant Industry of the United States Department of Agriculture and the Ohio Agricultural Experiment Station. The mineral analyses of corn plants reported by Latshaw and Miller [4] served as a general guide to the minerals to be included, though no attempt was made to approximate the relative percentages of each element. Each medium was infested with 100 or more newly hatched corn borer larvae. A small quantity of food material was spread about ¼ inch thick around the side walls of glass crystallizing dishes, which served as rearing chambers. The rearing chambers were 2 inches in diameter by 1½ inches

[1] Received for publication March 18, 1942.
[1] Grateful acknowledgment of assistance is made to W. A. Baker, in charge of European corn borer research, under whose leadership these studies were initiated and conducted.
[3] BOTTGER, G. T. PRELIMINARY STUDIES OF THE NUTRITIVE REQUIREMENTS OF THE EUROPEAN CORN BORER. Jour. Agr. Res. 60: 249–257. 1940.
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deep, and covered with an 80-mesh copper-screen disk, which was held in place with a ¼-inch rubber band. The food in each dish was infested with 20 individuals, and the number was gradually reduced as growth of the larvae increased their space and food requirements. The larvae were transferred to fresh food and a clean receptacle every second or third day during a 40-day test period. After the tenth day all larvae were weighed individually when they were transferred to fresh food.

Numerous preliminary tests, utilizing various materials as carriers and binders for the essential nutritive elements, were conducted before a medium possessing the proper physical qualities was formulated. Cellulose, supplemented with a small quantity of some cereal preparation such as cornstarch, corn meal, or oatmeal, and in some tests gelatin, as a binder, constituted the carrier or foundation for the nutritive elements. While such media sufficed to maintain life and foster a certain degree of growth in a few of the test larvae, in edibility and moisture-retaining ability they were not satisfactory. Furthermore, it was desired to eliminate all cereal matter from the media because of chemical complexities. Agar was one of the materials tested independently as a carrier and temporarily discarded, but its nonnitrogenous character, water-carrying capacity, and ability to remain congealed at incubator-room temperatures again suggested its possible utility. Accordingly, hot liquid agar was mixed with pulverized cotton in an effort to form a more satisfactory carrier. The carrier finally used was made by dissolving shredded agar in hot water and mixing it with cellulose that had been soaked in water. The material was then placed in a water bath and boiled 10 minutes. Known quantities of the dry nutritive elements and fats were then added, and the medium was stirred or beaten vigorously until it was of a homogeneous texture. As the heat treatment might reduce the dietary properties of some of the vitamins, particularly B_1, the composition was allowed to cool to near room temperature before they were added.

Various combinations of moisture, carbohydrates, proteins, fats, and vitamins were used in the 20 tests made with this carrier (table 1). The quantity of total mineral matter was also varied, but the same compounds in the same proportion (table 2) were used in all except formulas B–6, B–10, and B–14, in which the minerals occurring naturally in the cereal component of the media supplied these elements.

The larvae used in the tests were progeny of field-run moths of the mixed races that now inhabit the Toledo locality; i. e., both single- and multiple-generation borers and hybrids of the two strains. Mature corn borer larvae collected from a single field in the fall were isolated and kept in cold storage at 38°–40° F. until 3 to 4 weeks prior to the start of an experiment. They were then placed in an incubator at 80° under moisture conditions suitable for pupation and moth emergence. Larvae just hatched from eggs laid by the moths were employed for infesting all materials tested.

The tests were conducted in an incubator room in which a constant temperature of 80°±1° F. and a minimum relative humidity of 70 percent were maintained.

TABLE 1.—*Synthetic food formulas tested in the laboratory for rearing larvae of the European corn borer*

TABLE 1.—*Synthetic food formulas tested in the laboratory for rearing larvae of the European corn borer*

Formula	Water	Cellulose	Agar	Proteins			Carbohydrates			Fat	Mineral	Vitamins (International units)				
				Peptone	Casein	Zein	Glucose	Sucrose	Starch			A	B_1	C	D	E
	Cc.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*					
B–1	90	3.5	3.0	0.02	2.0	1.0	2.0	1.0	0	0.3	0.3	90	150	0	127.5	[1] ++
B–2	90	3.0	3.0	.04	2.0	0	2.0	1.0	0	.3	.3	90	150	250	627.5	[1] +++
B–3	90	3.5	3.0	0	2.0	0	2.0	1.0	0	.3	.3	900	0	0	225	[1] +
B–4	85	3.5	3.0	.02	2.0	0	2.0	1.0	0	0	.3	90	0	0	127.5	0
B–5	75	3.5	2.0	.01	1.5	0	1.5	1.5	0	1.0	.2	0	150	0	0	0
B–6	70	3.0	2.0	.03	[2] 5.2	0		[3] 13.6		1.4	1.3	0	0	0	0	0
B–7	70	3.5	1.0	.02	2.5	0	2.5	2.5	0	1.0	.6	0	150	0	0	0
B–8	85	3.5	3.0	0	2.0	0	2.0	1.0	0	.3	.3	0	150	250	0	[1] +
B–9	85	3.5	3.0	.02	2.0	0	2.0	1.0	0	.3	.3	0	0	0	0	0
B–10	72	3.0	2.0		[4] 3.2			[3] 13.6		1.4	1.3	0	0	0	0	0
B–11	85	3.5	3.0	.02	2.0	0	2.0	1.0	0	0	.3	150	0	0	0	0
B–12	85	3.5	3.0	.20	0	2.0	2.0	1.0	0	.2	.3	90	150	0	1,627.5	[1] +
B–13	75	2.5	2.5	0	2.0	0	2.0	1.0	0	.3	.3	1,200	150	200	300	[1] ++
B–14	75	3.0	2.0		[4] 3.2			[3] 13.6		1.4	1.3	0	0	0	0	0
B–15	70	2.5	0	.02	1.0	0	1.5	1.0	1.0	0	.5	0	150	0	0	0
B–16	70	2.5	2.0	.03	1.0	1.5	2.0	0	0	.3	.3	90	150	0	127.5	0
B–17	70	3.5	2.0	.01	1.5	0	1.5	1.5	1.0	.5	.3	0	0	0	0	0
B–18	85	3.0	3.0	0	0	2.0	2.0	1.0	0	0	.3	0	0	0	0	0
B–19	90	3.0	3.0	0	0	2.0	2.0	1.0	0	.3	.3	0	0	0	0	0
B–20	85	3.0	3.0	0	0	0	0	0	0	0	0	0	0	0	0	0

[1] Vitamin E was supplied in wheat-germ oil, indicated as fat, concentration unknown.
[2] Includes 2.0 gm. of casein plus 3.2 gm. of cereal proteins.
[3] Total carbohydrates as supplied in cereal component of these media.
[4] Total proteins as supplied in cereal component of these media.

TABLE 2.—*Inorganic mineral salts in synthetic food media tested for rearing larvae of the European corn borer*

Compound	Formula	Proportionate amount
Potassium dihydrogen phosphate	KH_2PO_4	1 gm.
Calcium sulfate	$CaSO_4 \cdot 2H_2O$	1 gm.
Ferric chloride	$FeCl_3 \cdot 6H_2O$	1 gm.
Magnesium sulfate	$MgSO_4 \cdot 7H_2O$	1 gm.
Copper ammonium hydroxide	$Cu(NH_3)_4(OH)_2$[1]	0.13 ml.[1]

[1] A saturated solution of this compound was prepared by bringing together ammonium hydroxide and cuprous oxide. The copper salt was added in excess of ammonium hydroxide.

PRESENTATION OF RESULTS

The corn borer was reared to maturity on an entirely synthetic food medium and so far as the author knows, this is the first instance of a phytophagous insect being so reared. Although these tests were designed primarily to determine whether the insect could be reared to maturity on a synthetic food medium, rather than to show the specific influences of the various elements utilized, the results do indicate the possibilities of this approach to studies of insect-nutrition requirements and provide general interpretations of the function of some of the more important constituents of the media.

Average percentages of survival at the end of 30 days, pupation at the end of 40 days, and weights of test larvae 20, 30, and 40 days old, reared on 20 samples of synthetic food media, are presented in table 3. The data are arranged to conform with the descending order of weights of 30-day-old larvae.

TABLE 3.—*Larval survival at end of 30 days, average weights of larvae as a result of feeding for 20, 30, and 40 days, and total pupation of surviving corn borer larvae at the close of the 40-day test period*

Formula	Average larval survival at end of 30 days	Average weight per larva at end of—			Total pupation of larvae surviving at end of 40 days
		20 days	30 days	40 days	
	Percent	*Milligrams*	*Milligrams*	*Milligrams*	*Percent*
B-1	8.1	22.4	81.5	83.8	9.2
B-2	14.0	32.7	72.0	81.3	16.7
B-3	13.0	24.0	69.1	62.6	25.0
B-4	15.0	21.3	65.3	69.1	0
B-5	4.2	15.6	61.4	79.5	20.0
B-6	8.0	36.7	58.5	74.3	23.5
B-7	11.8	18.2	56.4	61.5	0
B-8	17.2	22.6	54.0	64.0	0
B-9	36.0	31.9	47.6	----	30.0
B-10	3.3	36.0	46.4	88.7	0
B-11	20.0	21.7	42.4	55.0	0
B-12	11.3	18.0	38.2	----	----
B-13	6.0	18.2	36.5	61.5	25.0
B-14	7.2	28.0	34.7	38.0	0
B-15	11.0	15.5	33.3	42.8	0
B-16	21.0	6.9	25.0	38.6	12.5
B-17	.4	4.0	22.2	89.0	0
B-18	4.5	4.6	17.8	23.5	0
B-19	1.5	4.1	15.5	18.0	0
B-20	0	----	----	----	0

While it is indicated that nutrition has some influence on the number of generations produced, all the differences in percentages of pupation between lots of test larvae herein reported are not due solely to variation in nutrition. The studies show that the growth rate and the inherent size limitation of the insect are closely related to the character that influences the number of generations produced. Generally, the multiple-generation larvae never attained so great a weight as the single-generation larvae, which grow more slowly. Of those larvae that survived, as many as 30 percent pupated in one test and 20 percent or more in four other tests.

DISCUSSION OF RESULTS

In general, the most satisfactory synthetic media for rearing larvae of the European corn borer to a physiological maturity that will permit normal pupation were formulated approximately as follows: Cellulose 3.5 gm., agar 3 gm., casein 2 gm., glucose 2 gm., sucrose 1 gm., total mineral salts 0.35 gm., fat 0.3 to 1.0 gm., and water 85 cc. The pH of such media averaged about 4.5 immediately after they were mixed, but it was somewhat lower after they had stood in the incubator room 24 hours or more. Media in which zein was employed as the sole source of proteins underwent greater changes in pH (lower readings) than did media in which casein was used. Lower pH readings were usually associated with higher larval mortality and relatively low weights of those larvae that did survive.

EFFECT OF NUTRIENTS ON LARVAL DEVELOPMENT

As many as 36 percent of corn borer larvae nourished entirely on a synthetic food medium survived to maturity and attained near-normal weights during a 30- to 40-day test period. Larval survivals of about 20 percent are obtained on corn under average field conditions and from 30 to 60 percent on favorable food under laboratory conditions.

The development of single-generation larvae confined to synthetic foods was retarded as compared with that of larvae reared at the same time and under the same environmental conditions on fresh green plant food. Likewise, the time prior to pupation of multiple-generation larvae when reared on synthetic foods was 3 or more days longer than that generally required when reared on green plant tissue.

Low survivals resulting from formulas B–10 and B–17 are probably due to factors other than nutrition, since the few surviving larvae attained satisfactory weights. The nonsurvival on B–20 as early as the tenth day after infestation was due to lack of nutritive substance, since the cellulose-agar base was unsupplemented for this check test.

Proteins

Low survivals, low weights, and nonpupation of surviving larvae on B–18 and B–19 are attributed to incompatibility of zein with other components of the media and to the lysine and other amino acid deficiencies peculiar to this protein. Superiority of casein to zein is indicated. It is known that casein contains more of the amino acids essential to the nutrition of higher animals than does zein, and it appears highly probable that other proteins present in the corn

plant and grain, which supply such amino acids as lysine, tryptophane, and histidine, are essential to the nutrition of the corn borer. Although casein is the best protein tested to date and serves as a rich source of lysine and most of the other dietary-essential amino acids, it is deficient in cystine, necessary for supporting growth in rats,[5] and this deficiency may have been largely responsible for the dwarfness among the borers supplied solely with this source of protein, as compared with those reared on plant tissue. Peptone employed as a supplement to either zein or casein appeared to stimulate feeding and subsequent growth of the test larvae. The legume proteins contain relatively large amounts of lysine, tryptophane, and histidine, in marked contrast to those of corn, a fact which probably materially contributes to the nutritional adaptiveness of beans and peas for successfully rearing the corn borer in the laboratory.

Carbohydrates

A 2-to-1 ratio of glucose to sucrose seems to meet the carbohydrate requirements of the borer. Absence of biological response to the inclusion of cornstarch in the food media is in full accord with the results of enzyme tests reported by Bottger,[6] in which the borer's inability to digest cornstarch was indicated by the negative reaction of the tests for amylase, the starch-splitting enzyme.

Fats

Fat requirements of the borer cannot be established on the basis of results from these tests, but nearly all the more satisfactory media contained some fat either as a carrier for vitamins or as an independent constituent. Fats were supplied in the form of corn oil, wheat-germ oil, and halibut-liver oil.

Minerals

Previous tests indicated that inclusion of several of the minerals found in corn greatly improves the nutritive qualities of synthetic media for the borer. Mineral salts were therefore included in all media tested. The buffering effect of certain minerals was of value in neutralizing the acidity attending some other components of the media. The inclusion of copper ammonium hydroxide also appeared to improve the physical properties of the media, thereby aiding the borer to spin its silken cocoon, or feeding web, which seems to be essential for normal feeding. Sodium chloride was included among the minerals employed in some earlier tests, but omitted in the series herein reported because it did not appear to improve the nutritive qualities of the media and because its concentration in the corn borer's natural host plant is believed to be relatively low. Latshaw and Miller [7] do not mention sodium in their report on the elemental composition of the corn plant.

[5] Sherman, Henry C. Chemistry of food and nutrition. Ed. 5, completely rewritten, 640 pp., illus. New York. 1937. See pp. 71–74.
[6] See footnote 3.
[7] See footnote 4.

Vitamins

There is some indication that vitamins A, B_1 (thiamin chloride), and possibly E, improved certain media. However, when protein was supplied in the form of casein or peptone and constituted 3 percent or more of the total constituents of the media, the vitamins did not appear to be essential for growth and development of the corn borer. No particular biological response to either vitamin C or vitamin D was evident.

Effect of Synthetic Food on the Metamorphosis and Reproduction of the Corn Borer

The length of the pupal stage of insects reared on the more favorable synthetic media was the same as for those reared on natural foods, and the moths emerged normally from such papae. Females reared on the synthetic media laid infertile eggs either when unmated or when given opportunity to mate with males reared on synthetic food. However, poor synchronization of emergence of the limited numbers of both sexes is believed to have prevented coitus. When a newly emerged male reared on a synthetic medium was mated with a virgin female reared on plant tissue, normal mating and deposition of fertile eggs resulted. Although attempts to mate females reared on synthetic media, which attained subnormal size in these experiments, with plant-reared normal-sized males were unsuccessful, it seems probable that such adults possessed the ability to reproduce under favorable circumstances. As female moths are normally larger than males, the reversal in size differences of the sexes in this attempted cross may have made the sexual union of these individuals physically impossible.

SUMMARY

Information accumulated from various nutrition studies of the European corn borer (*Pyrausta nubilalis* (Hbn.)) was used in conducting a series of tests for the purpose of rearing larvae to maturity on a synthetic food medium.

Twenty food media were formulated to approximate the chemical composition of green corn tissue. The media were immediately placed in glass rearing receptacles and infested with newly hatched larvae. The tests were conducted in a room-size incubator where a constant temperature of 80° ± 1° F. and a minimum relative humidity of 70 percent were maintained.

Since the test larvae were parented by field-run stock of the mixed races of the European corn borer now occurring in the vicinity of Toledo, Ohio, both single- and multiple-generation strains resulted. The expression of the single- or multiple-generation characters is probably influenced by nutrition, but all the differences in pupation between tests should not be attributed to nutritive effects.

As many as 36 percent of the corn borer larvae confined to synthetic food survived to maturity; in one test as many as 30 percent, and in four other tests 20 percent or more larvae pupated.

Superiority of casein to zein as a source of protein was indicated. Peptone employed as a supplement to either casein or zein appeared to stimulate both feeding and growth of larvae.

A 2-to-1 ratio of glucose to sucrose appeared to satisfy the carbohydrate requirement of the corn borer.

Fat requirements of the corn borer cannot be established on the basis of these tests, but most of the more satisfactory media contained some fat.

Inorganic mineral salts were included in all media. Previous tests have indicated that certain mineral salts are nutritionally beneficial to corn borer larvae and also tend to buffer the media against excessive acidity.

There was some indication that vitamins A, B_1 (thiamin chloride), and E are of nutritional value to the corn borer, particularly in the absence of a sufficiently high percentage of casein in the food medium.

The duration of the pupal stage was normal, and moths of both sexes emerged normally. Poor synchronization of emergence of the limited numbers of male and female moths is believed to have prevented normal mating and the deposition of fertile eggs.

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